



Updates from the CFSRE's NPS Discovery

Current Trends in Seized Drug Analysis – Webinar – Monday January 23, 2023

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INTRODUCTION

- **Center for Forensic Science Research & Education**

- Associate Director
 - Toxicology & Chemistry
- Program Manager
 - NPS Discovery

- **Thomas Jefferson University**

- Assistant Program Director
 - MS in Forensic Toxicology
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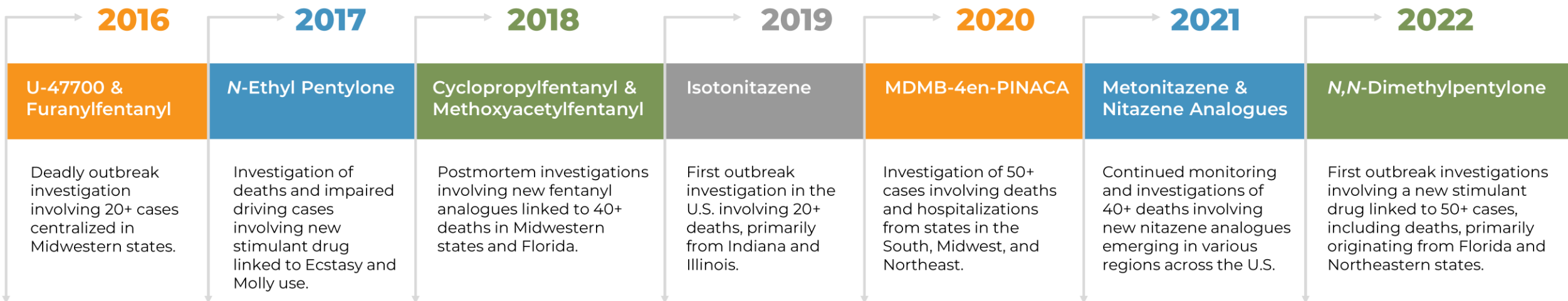




CFSRE'S NPS DISCOVERY

NPS DISCOVERY – THE CFSRE’S EWS

- Open-access drug early warning system (EWS)
 - Combine aspects of research & authentic cases
 - Analyze samples and generate data in-house
 - Develop a panel of high impact reports
 - Disseminate results and reports widely to stakeholders



NPS DISCOVERY – THE CFSRE’S EWS

2017

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

2018

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

2019

- Launched first NPS Discovery website to archive reports and data
- Began issuing *Public Alerts* to scientific stakeholders and professionals

2020

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

2021

- Greatly expanding program outputs (including several new report types)
- Library database expanded to encompass >950 drugs and NPS

2022

- Advancements in *Drug Checking* initiatives (purity testing, potency index)
- **Re-launch of NPS Discovery website** and development of *Year In Review*

2023

- Development of NPS naming & classification (collaboration with Cayman Chemical)
- Many more opportunities continuing to evolve !!!

Postmortem Toxicology

Impaired Driving

Emergency Department

Drug Materials

Drug Use Forums

Gray Market Sites

Additional Populations

[illegible][illegible]

2022

Figure 1: The report provides information and updated statistics about the emergence and biological and epidemiological characteristics (NPS) of the coronavirus SARS-CoV-2. The report also provides information on the epidemiological characteristics of the virus, including the number of cases, deaths, and hospitalizations.

Since 2019, NPS Discovery has identified 137 novel NPS discovered in the US and State of Texas. NPS identified represent the largest volume (Figure 2). In 2022, NPS discovery reported the discovery of 7 NPS for the first time.



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	7	0	7

Figure 3: Pie chart showing the distribution of NPS identified by year. The chart shows that 42% of NPS were identified in 2022, 35% in 2021, 15% in 2020, and 8% in 2019.



Year	Percentage
2019	8%
2020	15%
2021	35%
2022	42%

Since 2019, NPS Discovery has identified 259 NPS in forensic samples (Figure 4). NPS identified, variants, and COVID-19 relatedness represent the largest sub-titles observed. In 2022, 76 total NPS were detected (Figure 5).



Year	Percentage
2019	4%
2020	15%
2021	24%
2022	57%



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	76	0	76

In 2022, NPS Discovery observed more than 2,300 total NPS-detections within examined sample populations (Figure 6). A portion of more than 10,000 total NPS-detections are our program launched in 2019 (Figure 6).



Year	Novel NPS	Revised NPS	Total NPS
2018	1	1	2
2019	2	2	4
2020	3	3	6
2021	4	4	8
2022	76	0	76

Figure 7: Bar chart showing the number of NPS identified by year. The chart shows a steady increase from 2019 to 2022, with 2022 having the highest count at 76.



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	76	0	76

Figure 8: Line chart showing the number of NPS identified by year. The chart shows a steady increase from 2019 to 2022, with 2022 having the highest count at 76.



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	76	0	76

Figure 9: Bar chart showing the number of NPS identified by year. The chart shows a steady increase from 2019 to 2022, with 2022 having the highest count at 76.



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	76	0	76

Figure 10: Line chart showing the number of NPS identified by year. The chart shows a steady increase from 2019 to 2022, with 2022 having the highest count at 76.



Year	Novel NPS	Revised NPS	Total NPS
2019	1	1	2
2020	2	2	4
2021	3	3	6
2022	76	0	76



RECENT RESULTS AND OUTCOMES

EMERGENCE OF NPS IN THE U.S.

- Since 2018, NPS Discovery has reported **137** newly discovered NPS in the United States (Figure 1). **NPS opioids** remain the largest subclass (Figure 2). In 2022, NPS Discovery reported the discovery of **21** NPS for the first time.

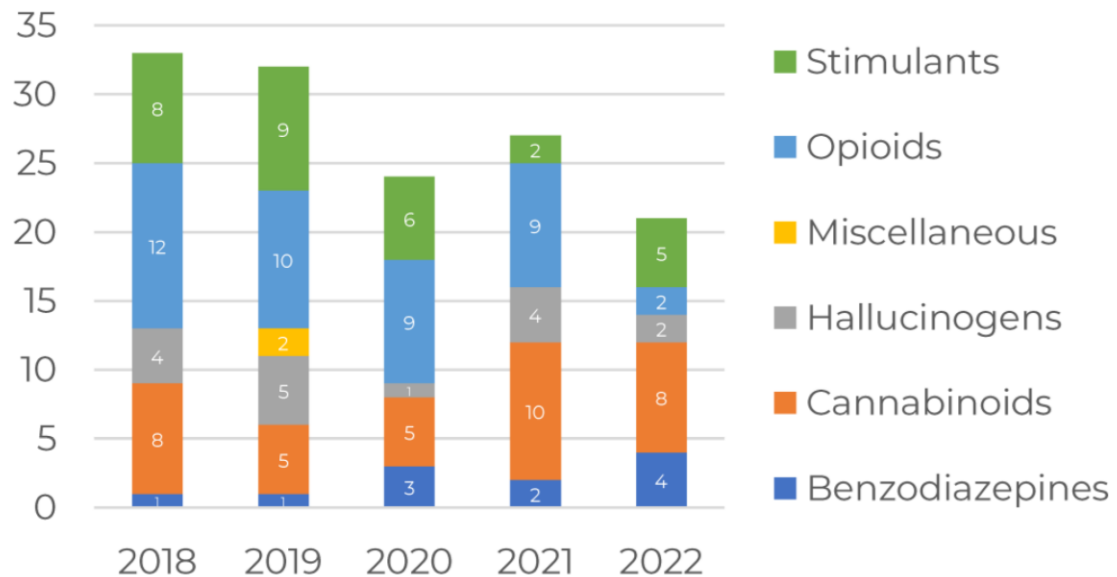


Figure 1: Newly discovered NPS reported for the first time since 2018.

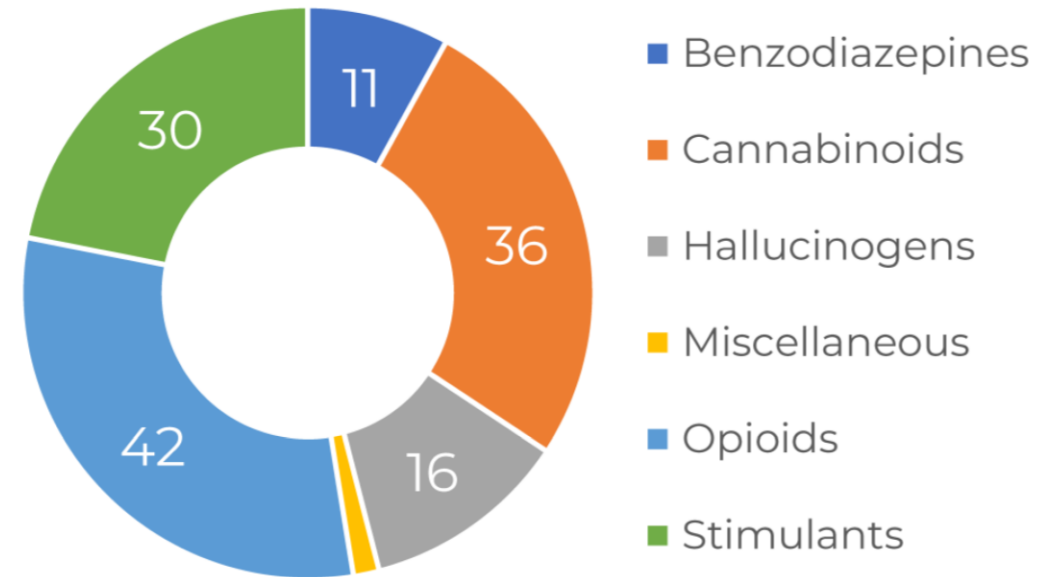


Figure 2: Breakdown by subclass of newly discovered NPS, 2018-2022.

LANDSCAPE OF NPS IN THE U.S.

- Since 2018, NPS Discovery has identified **218** NPS in forensic samples (Figure 3). **NPS opioids**, **stimulants**, and **cannabinoids** represent the largest subclasses observed. In 2022, **76** total NPS were detected (Figure 4).

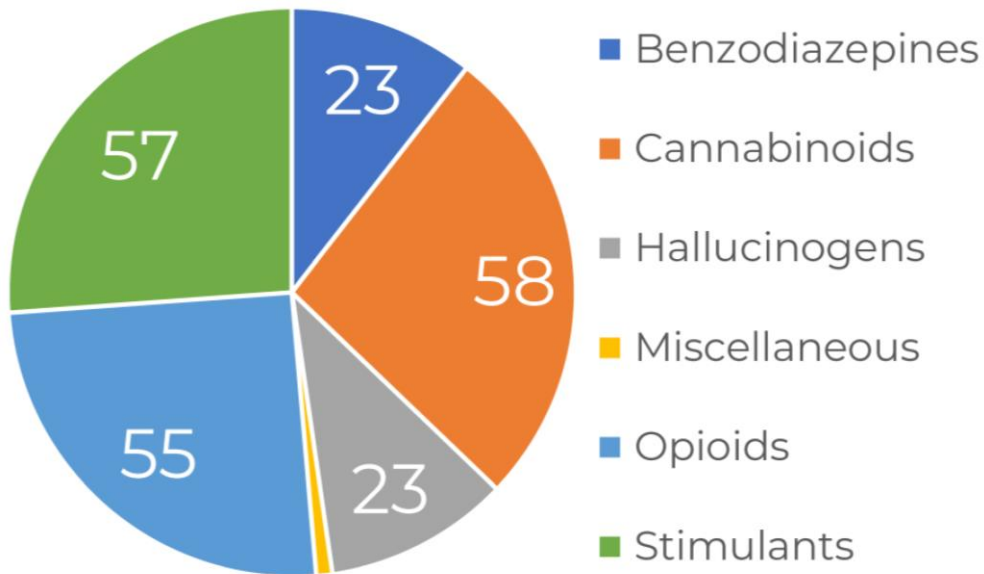


Figure 3: Breakdown by subclass of individual NPS detected, 2018-2022.

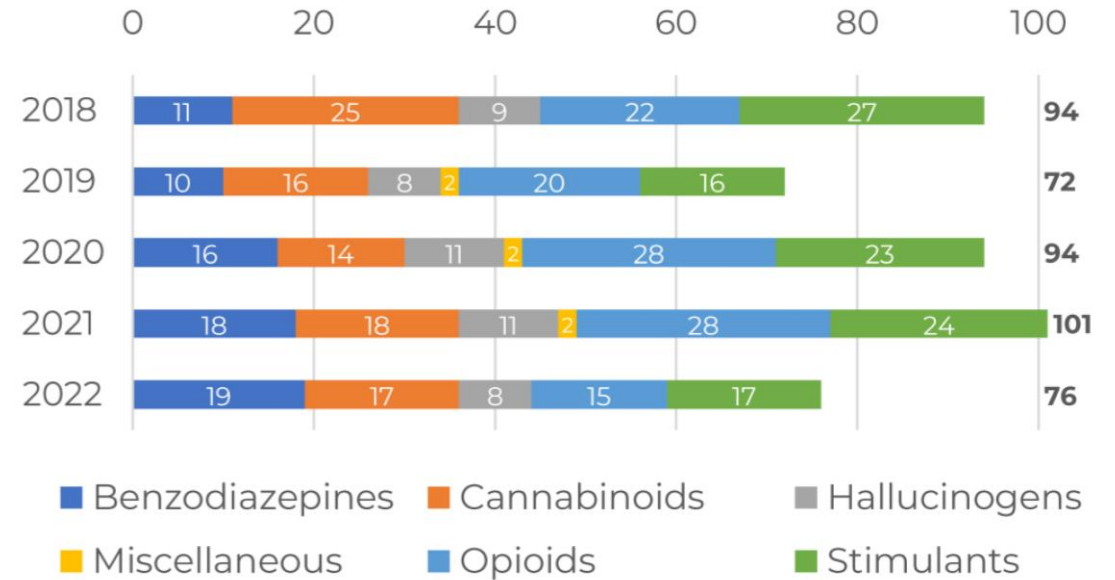


Figure 4: Individual NPS detected each year, cumulative since 2018.

PROLIFERATION OF NPS IN THE U.S.

- In 2022, NPS Discovery observed more than **2,200** total NPS detections within examined sample populations (Figure 5), a portion of more than **10,000** total NPS detections since our program launched in 2018 (Figure 6).

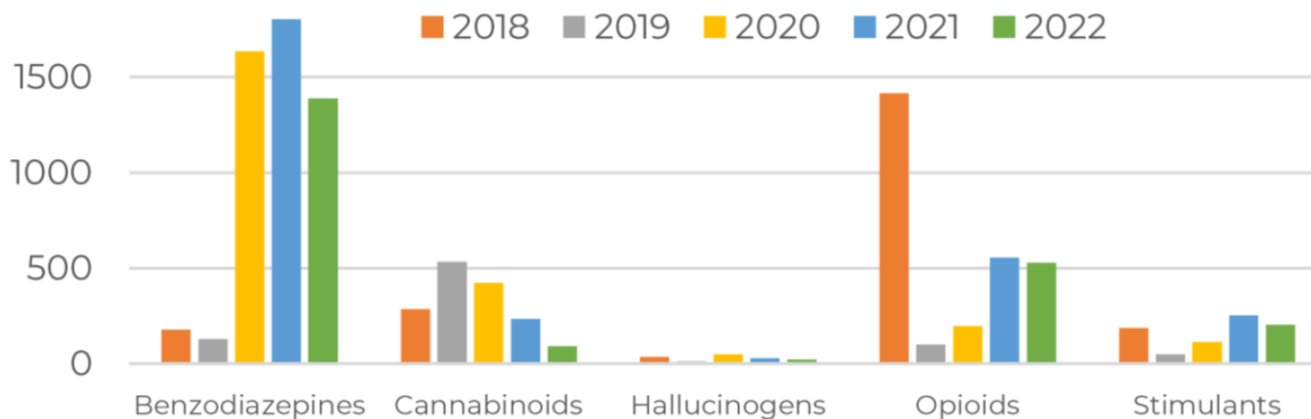


Figure 5: Total number of NPS detections among all samples analyzed since 2018.

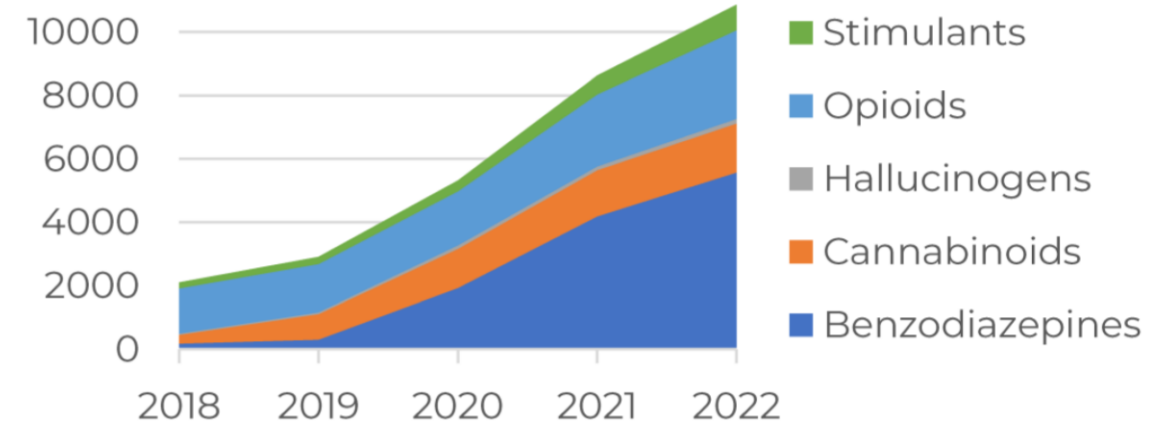
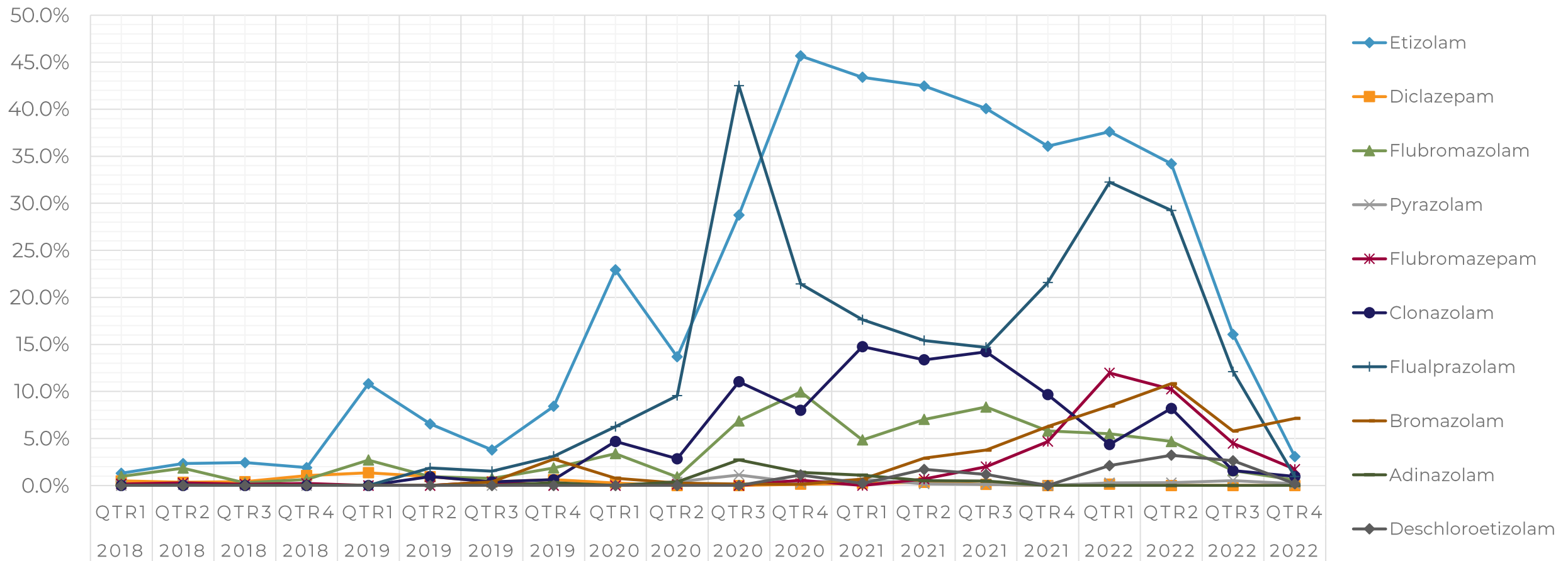


Figure 6: Cumulative number of NPS detections since 2018.

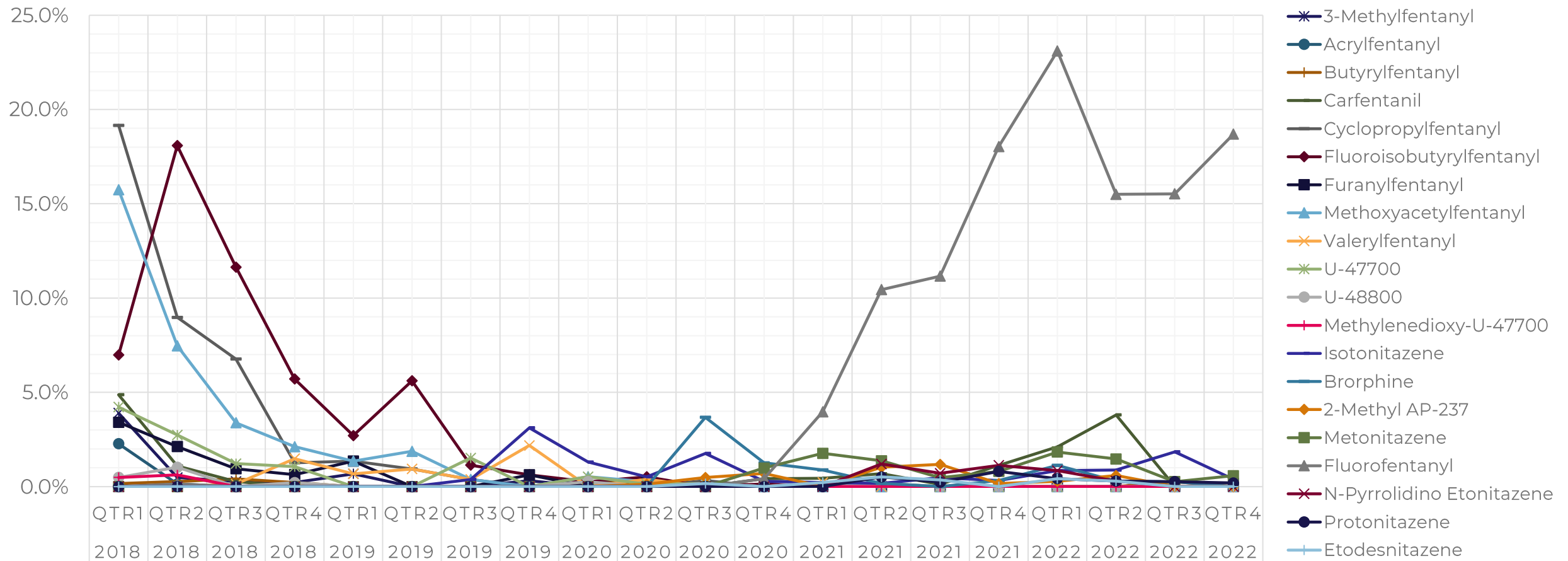
TRENDS FOR NPS SUBCLASSES IN THE U.S.

NPS Benzodiazepine Positivity in Toxicology Samples Analyzed at CFSRE By LC-QTOF-MS



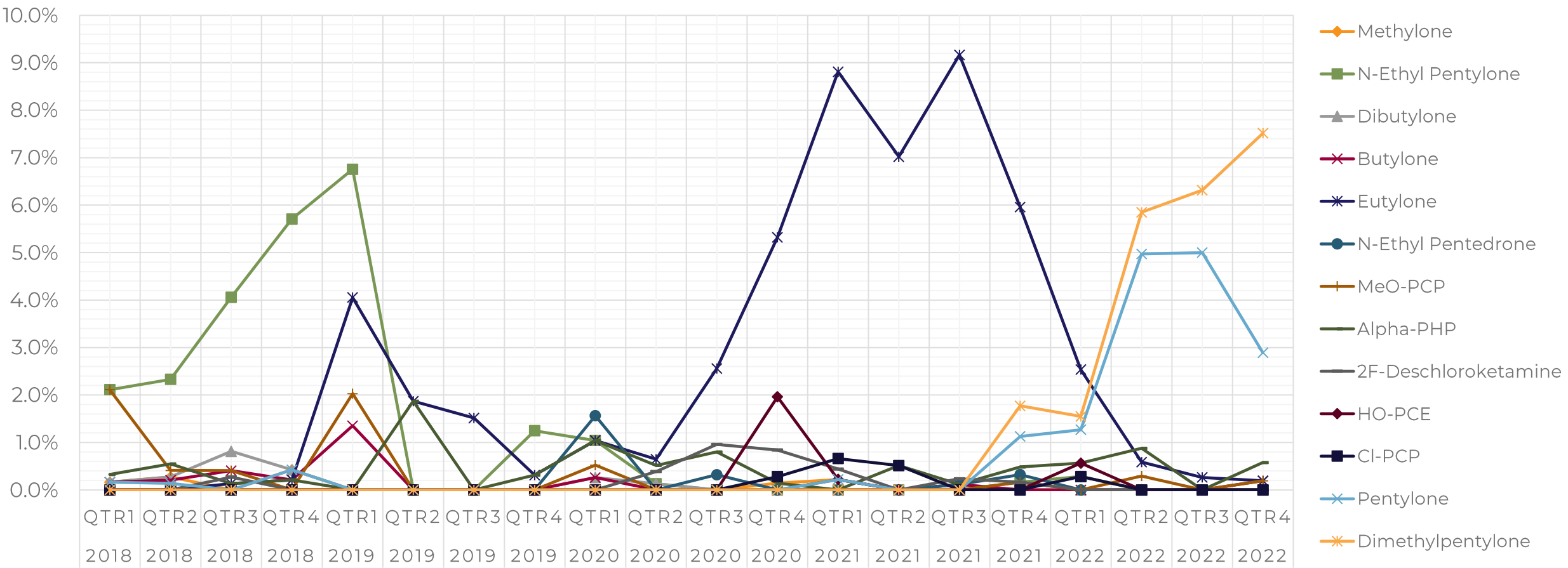
TRENDS FOR NPS SUBCLASSES IN THE U.S.

NPS Opioid Positivity in Toxicology Samples Analyzed at CFSRE By LC-QTOF-MS



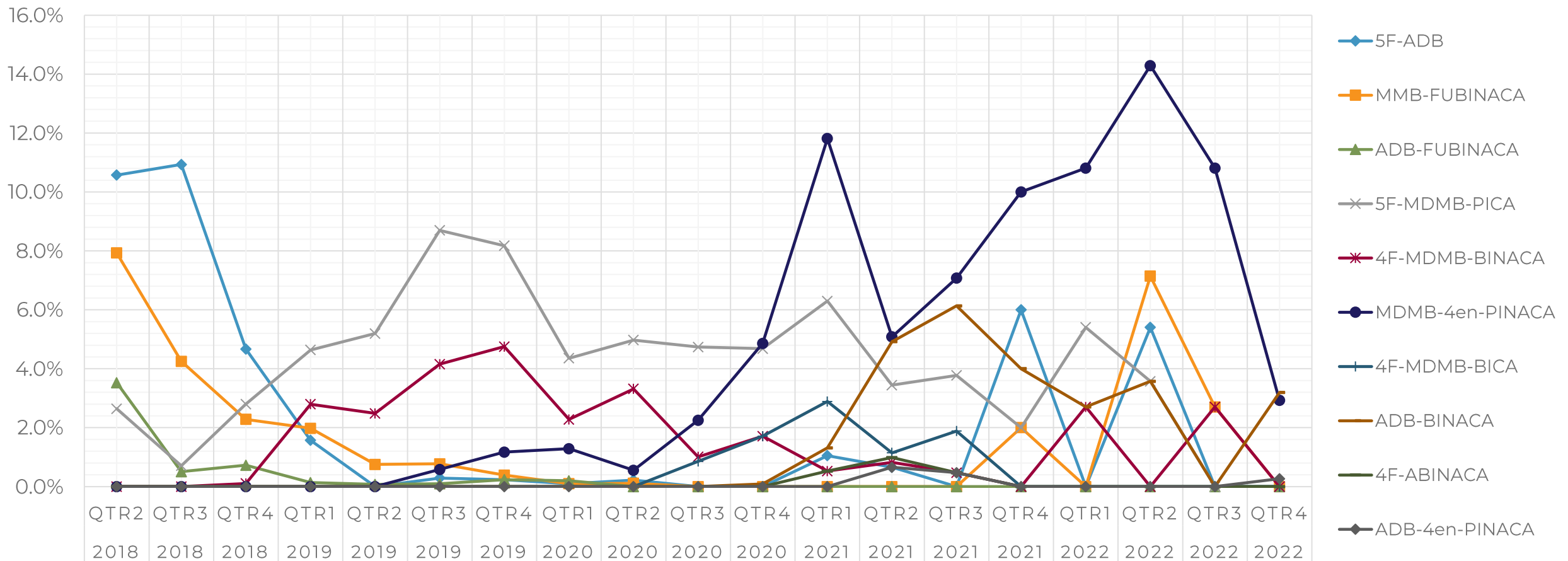
TRENDS FOR NPS SUBCLASSES IN THE U.S.

NPS Stimulant & Hallucinogen Positivity in Toxicology Samples Analyzed at CFSRE By LC-QTOF-MS



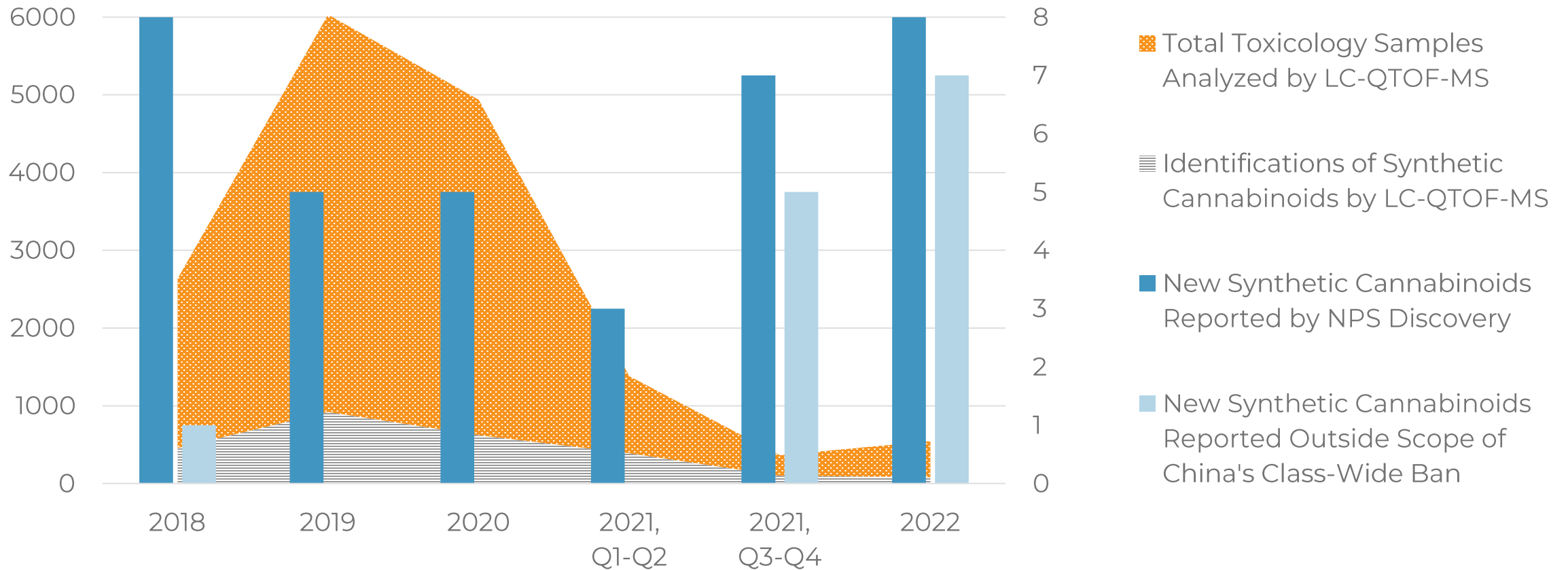
TRENDS FOR NPS SUBCLASSES IN THE U.S.

Synthetic Cannabinoid Positivity in Toxicology Samples Analyzed at CFSRE By LC-QTOF-MS



TRENDS FOR NPS SUBCLASSES IN THE U.S.

Nearly and all new **synthetic cannabinoids** fall outside the class-wide ban implemented in China and percent positivity has largely fallen off in the wake



PUBLIC ALERTS RECENTLY RELEASED BY NPS DISCOVERY

April 2022

Synthetic Stimulant Market Rapidly Changing as *N,N*-Dimethylpentylone Replaces Eutylone in Drug Supply Typically Sold as "Ecstasy" or "Molly"

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 stimulant *N,N*-dimethylpentylone.

Background: Synthetic stimulants are chemically manufactured drugs with sub-classifications based on their structural relation to amphetamine or cathinone. Synthetic stimulants, including substituted cathinone analogues (e.g., eutylone), can retain both stimulant and hallucinogenic properties, and can cause associated health risks. Synthetic stimulants are often prepared and distributed in powder, capsule, or tablet form, and may be sold as "Ecstasy", "Molly", or "MDMA" (3,4-methylenedioxymethamphetamine) on recreational drug markets. In the United States (U.S.), synthetic stimulants have been associated with adverse effects and linked to cardiac effects resulting in death. Adverse effects can include hyperthermia, dehydration, arrhythmias, hallucinations, and serotonin syndrome.

Summary: In 2020 and 2021, the substituted cathinone **eutylone** was the most commonly encountered synthetic stimulant to appear in forensic casework, despite the drug being considered federally scheduled as an isomer of pentylone since March 2017 according to the U.S. Drug Enforcement Administration (DEA). In September 2021, eutylone was recommended for international control. It is this notice that likely created a shift in the NPS drug market, which would later be noted by declining eutylone positivity and increasing *N,N*-dimethylpentylone positivity. *N,N*-Dimethylpentylone was first identified in toxicology samples in the U.S. in Q3 2021, marking the initial insurgence of this drug into the supply and the beginning of its proliferation. To date, *N,N*-dimethylpentylone has been identified in 32 toxicology cases, including antemortem and postmortem investigations, in addition to drug material cases. *N,N*-Dimethylpentylone is not explicitly scheduled in the U.S., however, it could be considered an isomer of *N*-ethyl pentylone (Schedule I). Of note, pentylone is a metabolite of *N,N*-dimethylpentylone.

Case Breakdown

Case Type:

- Postmortem (n=26)
- DUID (n=1)
- Unknown (n=5)

Date of Collection:

- August 2021 to March 2022

Other Notable Findings:

- Pentylone (n=23)
- Eutylone (n=5)
- Methamphetamine (n=11)
- Fentanyl / Opioids (n=13)
- No Other Drugs (n=6)

Geographical Distribution of *N,N*-Dimethylpentylone in the U.S.

Conc. in Postmortem Blood [ng/mL] (n=5)

<i>N,N</i> -Dimethylpentylone	Pentylone
Mean (s.d.)	270 ± 490
Median	87
Range	33 - 970

Recommendations for Public Health

- Implement surveillance for rapid identification of drug use and 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 people who use stimulant/hallucinogen drugs.
- Raise awareness about the risks and dangers associated with synthetic stimulant use.

Recommendations for Laboratories

- Utilize analytical data available publicly for the identification of *N,N*-dimethylpentylone.
- Utilize non-targeted testing protocols or develop sensitive and up-to-date testing procedures.
- Prioritize testing of drug material samples.
- Share data on synthetic stimulant identifications with local health departments, forensic scientists, and related communities.

Recommendations for Clinicians

- Become familiar with the signs and symptoms of synthetic stimulant use (e.g., agitation, hallucinations, excitement, elevated pulse, arrhythmias, serotonin syndrome).
- Be mindful that recreational drugs have limited quality control, containing undeclared substances that impact expected clinical effects or findings.
- Be aware that concentrations of synthetic stimulants in biological specimens can vary; however, GC-MS sensitivity may be adequate.
- Consult about the potential harms of "Ecstasy", "Molly", and "MDMA" products and use.

Recommendations for MEs & Coroners

- Test for new synthetic stimulants and their biomarkers in suspected anted-ant-related cases.
- Be aware that ELISA screening for synthetic stimulants may not be specific or specialized for the newest generation of drugs; consider mass spectrometry-based screening.
- Be aware that concentrations of synthetic stimulants in biological specimens can vary; however, GC-MS sensitivity may be adequate.

Rapid NPS Testing Now Available:

If your agency requests synthetic stimulant toxicity with an identifiable cause or your jurisdiction is detecting an increase in overdose patients requiring analgesic testing, contact NPS Discovery at the Center for Forensic Science Research and Education (CFSRE), a non-profit organization in collaboration with local and federal agencies that can provide rapid testing after novel drug outbreaks in the United States.

Website: www.npsdiscovery.org Email: npsdiscovery@cfsre.org

June 2022

Bromazolam Prevalence Surging Across the United States Driven In Part by Increasing Detections Alongside Fentanyl

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 benzodiazepine bromazolam.

Background: NPS benzodiazepines, referred to as novel or designer benzodiazepines, are synthetically manufactured drugs with unknown biological effects and health risks. NPS benzodiazepines are of public health and safety concern due to the potential for 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 their presence in forensic cases of high importance, paired with increasing concerns over combinations of benzodiazepines with opioids, colloquially known as "benzo-dope". NPS benzodiazepines can appear in various drug preparations, including powders, tablets, liquids, and blotter.

Summary: Bromazolam first emerged in the recreational drug supply in 2016 (Europe) and 2019 (United States). Bromazolam was first synthesized during medicinal drug development in the 1970s but never approved for therapeutic use in the United States. Bromazolam is the brominated counterpart to the chlorinated drug alprazolam. Bromazolam has been linked to adverse events resulting in hospitalization and death. Bromazolam is commonly reported in combination with other drugs, including the opioid fentanyl. To date, bromazolam has been identified in more than 250 toxicology cases submitted to NMS Labs, including both antemortem and postmortem investigations. Bromazolam has been identified in more than 190 toxicology samples tested at the Center for Forensic Science Research and Education (CFSRE), displaying an increase in positivity from 1% in Q1 2021 to 13% in Q2 2022. More significantly, co-detections with fentanyl have increased in recent months to more than 75% for bromazolam positive samples. Bromazolam has also been confirmed in counterfeit benzodiazepine preparations at the CFSRE.

Bromazolam Blood Conc. (ng/mL)

Postmortem Investigation (n=236)

Mean (s.d.)	65 ± 79
Median	35
Range	2.1 - 670

Drug Impaired Driving (n=14)

Mean (s.d.)	61 ± 47
Median	36
Range	4.3 - 160

ALPRAZOLAM BROMAZOLAM

Bromazolam Cases and Positivity in the U.S. (Source: CFSRE)

Bromazolam Geographical Distribution in the U.S. (Source: NMS Labs, Feb. 2021 to May 2022)

Recommendations for Public Health

- Implement surveillance for rapid identification of drug use and overdose outbreaks, monitor geographical drug distribution and trends.
- Engage local poison centers and clinicians to assist with treatment of affected patients.
- Track demographics and risk factors for people who use benzodiazepines and opioids.
- Raise awareness about the risks and dangers associated with new benzodiazepine use.

Recommendations for MEs & Coroners

- Test for new benzodiazepines and their biomarkers in suspected benzodiazepine-related and opioid-related cases.
- Be aware that ELISA screening for new benzodiazepines may not be specific or specialized for the newest generation of drugs; consider mass spectrometry-based screening.
- Be aware that concentrations of new benzodiazepines 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 bromazolam.
- Utilize non-targeted testing protocols or develop sensitive and up-to-date testing procedures.
- Prioritize testing of drug material samples.
- Share data on benzodiazepine and opioid identifications with local health departments, forensic scientists, and related communities.

Recommendations for Clinicians

- Become familiar with the signs and symptoms of new benzodiazepine use (e.g., sedation, drowsiness, slurred speech, motor incoordination), with and without opioids.
- Be mindful that recreational drugs have limited quality control, containing undeclared substances that impact expected clinical effects or findings.
- Consult about the potential harms of benzodiazepine products (e.g., counterfeit tablets, pressed "Xanax" bars).
- Be mindful that drugs have limited quality control, containing undeclared substances that impact clinical effects or findings.
- Consult about the harms and dangers of synthetic opioid products and other drugs.

Rapid NPS Testing Now Available:

If your agency requests new benzodiazepine toxicity with an identifiable cause or your jurisdiction is noticing an increase in overdose patients requiring analgesic testing, contact NPS Discovery at the Center for Forensic Science Research and Education (CFSRE), a non-profit organization in collaboration with local and federal agencies that can provide rapid testing after novel drug outbreaks in the United States.

Website: www.npsdiscovery.org Email: npsdiscovery@cfsre.org

PUBLIC ALERT JAN 2023

NEW POTENT SYNTHETIC OPIOID—*N*-DESETHYL ISOTONITAZENE—PROLIFERATING AMONG RECREATIONAL DRUG SUPPLY IN USA

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*-desethyl isotonitazene.

BACKGROUND: Synthetic opioids (e.g., fentanyl, fentanyl analogues) are chemically manufactured drugs, often having unknown potency and adverse effects or health risks. Synthetic opioids are frequently mixed with more traditional opioids (e.g., heroin) and other drugs in unregulated drug markets creating additional risk and danger for people who use recreational drugs. Synthetic opioids may be distributed in powder or tablet form. In the United States (USA), an alarming increase in the number of deaths linked to synthetic opioid use has been reported. Primary adverse effects associated with synthetic opioid use are sedation and respiratory depression, leading to death.

SUMMARY: *N*-Desethyl isotonitazene is a new synthetic opioid bearing structural resemblance to isotonitazene and recently emergent nitazene analogues. *N*-Desethyl isotonitazene is dissimilar in chemical structure to fentanyl, the synthetic opioid most commonly encountered, but this subclass of new nitazene has been proliferating in the wake of the scheduling of fentanyl analogues. *N*-Desethyl isotonitazene is a known metabolite of isotonitazene; however, it has now emerged as a primary drug in its own right. Most nitazene analogues encountered retain opioid receptor activity and potency similar to or greater than fentanyl. In vitro pharmacological data show that *N*-desethyl isotonitazene is an active opioid agonist and is approximately 20x more potent than fentanyl. In December 2022, *N*-desethyl isotonitazene was first reported by NPS Discovery (Florida); however, first identifications were observed as early as September 2022. To date, seven drug material samples ("dope" powders) collected from the Philadelphia drug supply have tested positive for *N*-desethyl isotonitazene. In December 2022, the Philadelphia Department of Public Health issued an alert regarding the discovery of this new nitazene analogue in the city's drug supply. The toxicity of *N*-desethyl isotonitazene has not been examined or reported but recent association with overdoses among people who use drugs leads professionals to believe this synthetic opioid has the potential to cause harm and is of high public health concern.

TIMELINE — *N*-DESETHYL ISOTONITAZENE ...

- Identified in urine samples from a drug treatment program (PA).
- Identified in oral fluid samples collected from people who use drugs (PA).
- Identified in a counterfeit "X215" (oxycodone) round blue tablet (FL).
- Identified in "dope" samples alongside fentanyl, xylazine, and bromazolam (PA).
- Continues to be identified in "dope" samples among Philadelphia drug supply (PA).

2022 September October November December 2023 January

***N*-DESETHYL ISOTONITAZENE**

"DOPE" SAMPLES CONTAINING *N*-DESETHYL ISOTONITAZENE

LOCATION: Philadelphia, PA, USA

NUMBER OF SAMPLES: 7+

CONTENTS (PURITY RANGE):

- Xylazine (49% to 70%)
- Fentanyl (1.1% to 5.1%)
- N*-Desethyl isotonitazene (0.05% to 0.4%)
- Bromazolam (trace to 2.5%)
- Flubromazepam (trace)
- para-Fluorofentanyl (trace)

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 other drugs on board or precipitation of withdrawal, which may be more severe with faster onset.
- Be mindful that drugs have limited quality control, containing undeclared substances that impact clinical effects or findings.
- Consult about the harms and dangers of synthetic opioid products and other drugs.

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.
- 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 other drugs on board or precipitation of withdrawal, which may be more severe with faster onset.
- Be mindful that drugs have limited quality control, containing undeclared substances that impact clinical effects or findings.
- Consult about the harms and dangers of synthetic opioid products and other drugs.

RECOMMENDATIONS FOR LABORATORIES

- Utilize analytical data available publicly for the identification of *N*-desethyl isotonitazene 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 and novel drugs.
- Prioritize analytical testing of drug materials obtained from drug overdose scenes during death investigations.
- Share data on synthetic opioid drug seizures with local health departments, medical examiners, coroners, and related communities.

RECOMMENDATIONS FOR MEDICAL EXAMINERS & CORONERS

- Test for new synthetic opioids and their biomarkers (if known) in suspected opioid overdose cases.
- Be aware that ELISA screening for synthetic opioids is not specific or specialized for the newest generations of drugs—Mass spectrometry-based screening is necessary.
- Be aware that concentrations of synthetic opioids in biological specimens can vary and GC-MS sensitivity may not be adequate.
- Consult with forensic toxicologists about novel opioid activity, potency, and association with overdose and/or death.

DRUG CHECKING AND POTENCY INDEX

Fentanyl Purity, Potency, & Synthesis

Real-Time Testing of Opioid Drug Products in the United States



WHAT IS FENTANYL?

Fentanyl is a synthetic opioid first synthesized in 1960. Fentanyl is used widely in medicine for the treatment of severe pain. Fentanyl is reported to be 50 to 100 times more potent than morphine. Like other opioids, fentanyl is a central nervous system depressant and in overdose scenarios can lead to a range of adverse effects, including sedation, respiratory depression, and in severe cases fatal overdose. The prevalence of fentanyl in the United States recreational drug supply has increased significantly since 2010, and is now the most commonly identified drug in the United States recreational drug supply. Recreational fentanyl (sometimes referred to as illicitly manufactured fentanyl) is the primary synthetic opioid identified in fatal drug overdoses, although there are increasing reports of fentanyl poly-drug overdoses (e.g., in combination with xylazine, benzodiazepines, stimulants). Recreational fentanyl is commonly ingested through various routes of administration, including intranasal, intravenous, and oral. The prevalence of fentanyl in the United States recreational drug supply is increasing, and its prevalence has thus far only increased despite various countermeasures.



Figure 1: Illustration of drug purity

WHAT ARE FENTANYL PRECURSORS, INTERMEDIATES, AND BYPRODUCTS?

Intenalty is a synthetic drug produced via chemical processes and reactions between starting materials or precursors. Intenalty can be chemically synthesized in different ways (Figure 9) using a variety of precursors. **Products** are defined as the starting molecules used for synthesis. During controlled pharmaceutical synthesis, careful selection of chemical reactions and clean-up processes ensure a correct pathway is followed, flowing through known **intermediates** to high purity final products (Figure 4). However, during clandestine synthesis, it is common that byproducts (or impurities) can appear. **Byproducts** are defined as unwanted molecules produced or left behind from chemical reactions of precursors or with intermediates. Byproducts are not the intended final drug product (e.g., fentanyl). Examples of precursors include 4-ANPP, 4-AP, benzylfentanyl, phenethylchloride, and NPP. Examples of intermediates include 4-ANPP, 4-ANRP, and benzylfentanyl. Examples of byproducts include 4-ANPP, phenethyl-4-ANPP, N-propionyl norfentanyl, and acetylchloride. To complicate matters, some precursors, intermediates, and byproducts may be used in the synthesis of other drugs. For example, 4-ANPP is used in the synthesis of 4-ANRP. Based on currently available information, the following table provides a summary of the chemical synthesis of fentanyl, precursors, intermediates, and byproducts are largely identical or related, only very low opioid activity with the exception of acetylchloride, making their presence in drug materials, especially in small quantities, of low pharmaceutical significance (although true toxicity of these chemicals remains unknown).

WHAT ARE PURITY AND POTENCY?

Purity is defined as the amount quantity of weight in a material or product. Purity is assessed on an individual drug basis and can be reported as a percent (%) or absolute weight (mg). For example, the purity of a 100 mg powder might be 30% (or 10 mg) for fentanyl, 30% (30 mg) for ylatrine, and 60% (60 mg) for mannitol (Figure 1). **Potency** is defined by the strength of effects the drug product can have in humans. Potency relates the purity of all pharmacologically active constituents. **Purity vs. potency** is best described by methamphetamine quantitative testing where purity is the amount of methamphetamine in a product and potency is more specifically the amount of *o*-methamphetamine. Herein relating to fentanyl, purity is the amount of fentanyl in a product and potency is the amount of *trans*-fentanyl (Figure 2). **Assay** is the method used to determine the purity of all species, including fentanyl, heroin and pentamorphant. Potency index is a relative measure of the relative pharmacological activity (EC₅₀), normalized, and expressed on a scale where a fentanyl potency of 10% potency represents a baseline unit of 1.

HOW IS PURITY DETERMINED?

Purity testing is determined through accurate quantitative analysis using **gas chromatography mass spectrometry (GC-MS)**. An external calibration model is developed using known quantities of drug reference materials to which the sample in question is compared. A defined weight (mg) of the drug product is measured followed by a series of specified dilutions in organic solvents and extraction of drugs from matrix (i.e., isolation from unwanted components). A final calculation is performed to determine purity as a percent (%) relative to the initial measured mass of an aliquot (or sub-sample).

HOW DOES PURITY TESTING SUPPORT HARM REDUCTION?

Qualitative fentanyl testing of drug products has been employed for many years in various forms for harm reduction purposes, from the employment of fentanyl test strips and FTIR in the fields to GC-MS and LC-MS assays in the laboratory. The results of qualitative testing for fentanyl are useful in certain scenarios; however, it has been observed that qualitative testing alone cannot answer more complex questions about drug products and their impacts (e.g., overdose surge, unexpected adverse effects). It has long been hypothesized that comprehensive, population-level quantitative fentanyl testing would serve as a better assessment of the drug supply – an observation that continues to be assessed and validated in countries outside the United States. Having fentanyl purity and potency data allows public health officials to better understand and assess the drug supply and use outcomes. Our preliminary data show that drug purity can vary between samples marked identically over time (Figure 2).

Objectives: A partnership between the Center for Forensic Health Research and Education (CFHRE) and the Philadelphia Department of Public Health (PDHP) has been formed to currently assess the top priority of public health emergencies, Zika. This initiative was established as a comprehensive effort examining various drug treatments and drug forms, in both qualitative and quantitative formats. The information and results reported herein represent a subset of the drug study and not its entirety.

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Figure 2: Authentic quantitative data from drug products with identical markings (stamp) collected in Philadelphia, Pennsylvania, USA, showcasing fluctuation in purity and potency.

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Fentanyl Purity, Potency, & Synthesis (CONT.)

DRUG PURITY VS. POTENCY INDEX

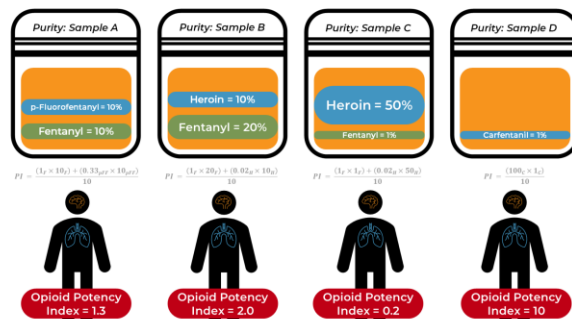


Figure 3: Illustration of drug purity (top) vs. potency index (bottom) with example calculations for opioids commonly encountered in the recreational drug supply

$$\text{Potency Index} = \frac{(PF \times Purity) + (PF \times Purity) \dots}{10} \quad (Eq.1)$$

CALCULATING POTENCY INDEX (PI)

The United States is in the midst of an overdose epidemic which is underlined by poly-drug use. Poly-drug combinations can be rooted in drug products containing, at times, more than one opioid. As quantitative drug purity data become available, scientists and public health officials need a comprehensive yet simple methodology to compare drug products. For this purpose, the **potency index** was developed. Potency index is a numeric value that takes into account the amount of a drug present (i.e., purity) and its relative potency, or potency factor (e.g., compared to fentanyl). The calculation is the sum of all similar pharmacologically active drugs present and is normalized to a comparator (e.g., fentanyl at 10% purity) for ease of understanding and utility. Potency index can be calculated for and applied to other drug classes, but herein is used as a representation of opioids.

Equation 1 can be applied to calculate Opioid Potency Index, where...

- PF is the Potency Factor compared to Fentanyl and is calculated as $EC_{50}^{Fentanyl}/EC_{50}^{opioid}$ as the μ opioid receptor.
 Examples of PFs: Fentanyl 1, Heroin = 0.02, pure Fluoranthene = 0.33, ortho-Fluoranthene = 1, Carfentanyl = 100.
 → Purity is the amount of drug in a specified sample and is expressed as a percent (e.g. 10%, 23%).
 → $\frac{1}{L}$ indicates that the numerator should be continued for all drugs in this case opioid present in 100.
 → The denominator is 10 → A Normalizing Factor applied so a sample of 10% fentanyl-only represents a Potency index of 1.
 → Potency index is reported to one decimal place (e.g., 0.9, 4.6, etc.) until the value exceeds 10.
 → Tolerance, dose, and other user factors are assumed to be constant at the individual level when assessing Potency index; however, it should be understood that these factors will influence inter-individual outcomes.

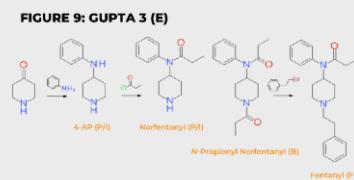
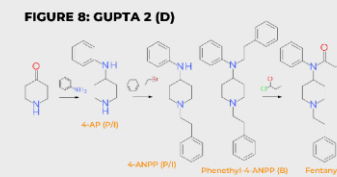
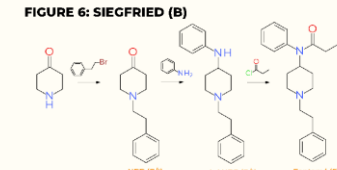
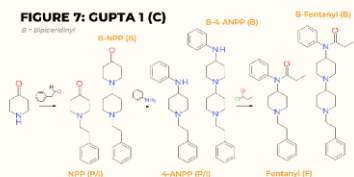
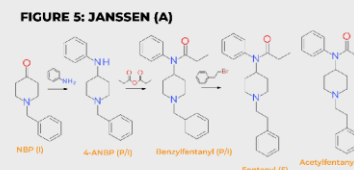


Fentanyl Purity, Potency, & Synthesis (CONT.)



Figure 4: Generic flow of chemicals during drug synthesis. Illustration shows when byproducts may be formed during the synthesis process.

FENTANYL SYNTHESIS PATHWAYS



Molecular Weight	Classification	Pathway/Class
4-ANBP	Procuror / Intermediate	A
4-ANPD	Procuror / Intermediate	B, C, D
4-AP	Procuror / Intermediate	D, E
Acetylcholinyl	Byproduct	A, Cytotoxic, Toxic
6-4-ANBP	Byproduct	C
Benzylcholinyl	Procuror / Intermediate	A
B-Formyl	Byproduct	C
N-BP	Byproduct	C
NBP	Procuror / Intermediate	E (at short term)
Nonformyl	Procuror / Intermediate	E
NDP	Procuror / Intermediate	B, C
N-Propionyl Nonformyl	Byproduct	C
N-Propionyl 4-ANBP	Byproduct	D

Note: All procurors, intermediates, and/or byproducts may not be listed for all patients.

Note: All precursors, intermediates, and/or byproducts may not be listed for all pathways.

CASE EXAMPLE – N-DESETHYL ISOTONITAZENE

New potent synthetic opioid proliferating among recreational drug supply in USA

- Latest nitazene analogue to emerge
- Approximately 20x more potent than fentanyl
- Two states: Florida and Pennsylvania
- Four sample types: pill, powders, urine, and oral fluid
- Severe overdose scenarios arising involving this drug



“DOPE” SAMPLES CONTAINING N-DESETHYL ISOTONITAZENE

LOCATION: Philadelphia, PA, USA

NUMBER OF SAMPLES: 7+

CONTENTS (PURITY RANGE):

- ▶ Xylazine (49% to 76%)
- ▶ Fentanyl (1.1% to 5.1%)
- ▶ N-Desethyl Isotonitazene (0.05% to 0.4%)
- ▶ Bromazolam (trace to 2.5%)
- ▶ Flubromazepam (trace)
- ▶ para-Fluorofentanyl (trace)

TIMELINE — N-DESETHYL ISOTONITAZENE ...

Identified in **urine samples** from a drug treatment program (PA).

Identified in **oral fluid samples** collected from people who use drugs (PA).

Identified in a **counterfeit “A215”** (oxycodone) round blue tablet (FL).

Identified in **“dope” samples** alongside fentanyl, xylazine, and bromazolam (PA).

Continues to be identified in **“dope” samples** among Philadelphia drug supply (PA).

2022

September

October

November

December

2023

January






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ACCESS NPS DISCOVERY REPORTS & JOIN OUR LISTSERV

NPS DISCOVERY



The CFSRE's NPS Discovery program is an open-access drug early-warning system (EWS) operating in the United States. Our evidence-based approach leads the development of high impact reports for real-time action among public health and safety stakeholders.

We are working in collaboration with forensic science, public health, emergency medicine, and criminal justice agencies to rapidly identify emerging drugs, also known as Novel Psychoactive Substances (NPS), associated with intoxications and adverse events. Our data and results are consolidated into reports and resources to allow for the rapid dissemination of information to colleagues and affected communities.

Stakeholders interested in receiving up-to-date information and notifications can join our [email listserv](#) (be sure to select the NPS Discovery check box at the bottom).

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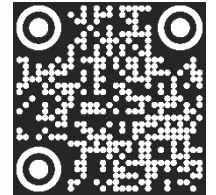
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