



Updates from the CFSRE's NPS Discovery

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

Alex J. Krotulski, PhD

Center for Forensic Science Research & Education (CFSRE)



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
 - Faculty / Lecturer

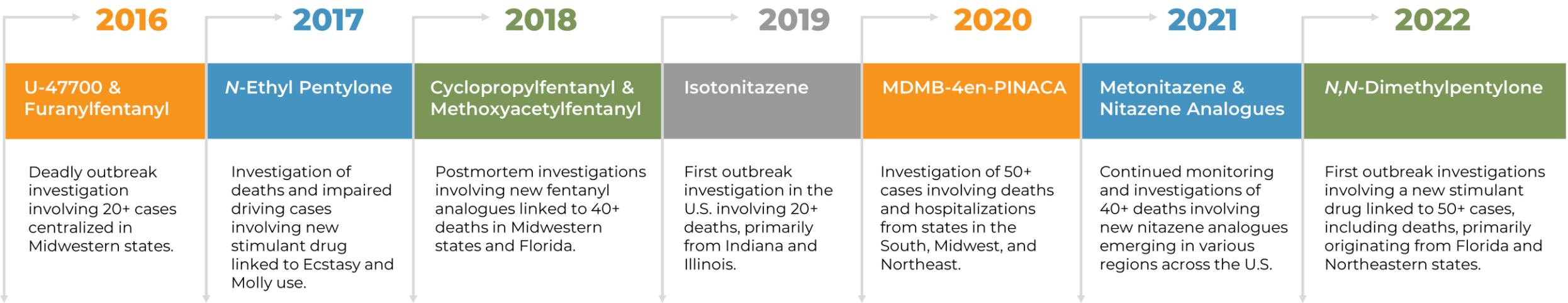
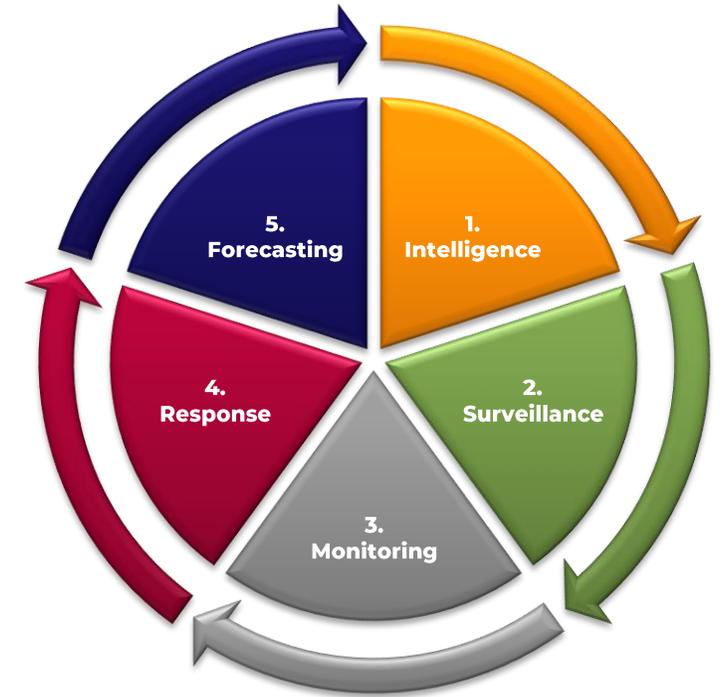




CFSRE'S NPS DISCOVERY

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 charactering 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

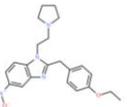
Gray Market Sites

Additional Populations

NPS DISCOVERY REPORTS → WWW.NPSDISCOVERY.ORG

N-Pyrrolidino Etonitazene
Sample Type: Biological Fluid

Latest Revision: May 13, 2021
Date of Report: May 13, 2021



I. GENERAL INFORMATION

IUPAC Name: 2-[3-(4-ethoxyphenyl)pyridin-5-yl]-1-(2-pyrrolidin-1-yl)ethanone
InChI String: CCOC1=CC=C(C=C1)C2=CN(C=C(C=C2)C3=CC=CC=C3)C(=O)CC4=CC=CC=C4N5CCCC5

CFR: Not Scheduled (09/2021)
CAS: Not Available
Synonyms: Etonitazepine
Source: NMS Labs - Toxicology Department

NPS Benzodiazepines in the United States

TREND REPORT Q4 2022

OVERVIEW: Most psychotropic substances (NPS) including NPS benzodiazepines continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS benzodiazepines have been implicated in an increasing number of adverse health events, marked by emergency room admissions and death investigations regularly when ingested in combination with opioids, resulting in a complex scope of analysis that is underlying, requiring comprehensive analytical methodologies and extensive research in identification.

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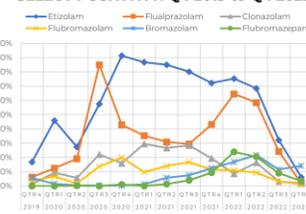
NPS in Q4 2022:

- 32% Opioids
- 32% Benzodiazepines and Stimulants/Hallucinogens
- 27% Synthetic Cannabinoids

NPS BENZODIAZEPINES IDENTIFIED

Phenazepam	2
Desallylflurazepam	2
Flurazepam	3
Desallylclonazepam	3
4-Cl-Deschlorazepam	3
Flurazepam	5
Clonazepam	9
Flurazepam	16
Bromazolam	39

SELECT POSITIVITY: Q4 2019 to Q4 2022



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

OVERVIEW: The objective of this research is to provide detailed guidance in developing an appropriate analytical scope of testing for forensic laboratories (NPS) in the United States based on current trends and emerging NPS. This report is based on information available to Q4 2022 and is subject to change along with the drug market.

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Recommended Scope for NPS Testing in the United States

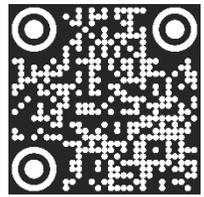
NPS SCOPE Q3 2022

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Toxic Fentanyl Study Group — Quarterly NPS Report

CLINICAL Q3 2022

OVERVIEW: This report provides an overview regarding comprehensive drug testing of clinical toxicology specimens collected from a representative cross-section of forensic laboratories in the United States (US).

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QUARTERLY REPORT — PHILADELPHIA, PA

DRUG CHECKING Q3 2022

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Fentanyl Purity, Potency, & Synthesis

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

WHAT IS FENTANYL?

WHAT ARE FENTANYL PRECURSORS, INTERMEDIATES, AND BYPRODUCTS?

WHAT ARE PURITY AND POTENCY?

HOW IS PURITY DETERMINED?

HOW DOES PURITY TESTING SUPPORT HARM REDUCTION?

NPS Discovery Toolkit

The Center for Forensic Science Research & Education

NPS DISCOVERY

N-Pyrrolidino Etonitazene

Stamp "X"

Stamp "X"

Stamp "X"

YEAR IN REVIEW 2022

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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.

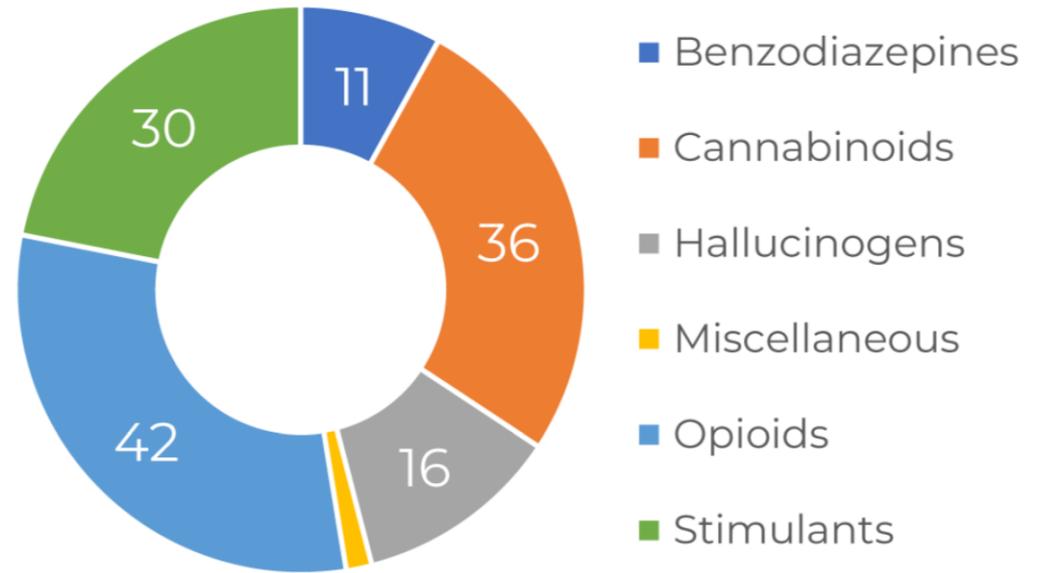


Figure 2: Breakdown by subclass of newly discovered NPS, 2018-2022.

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).

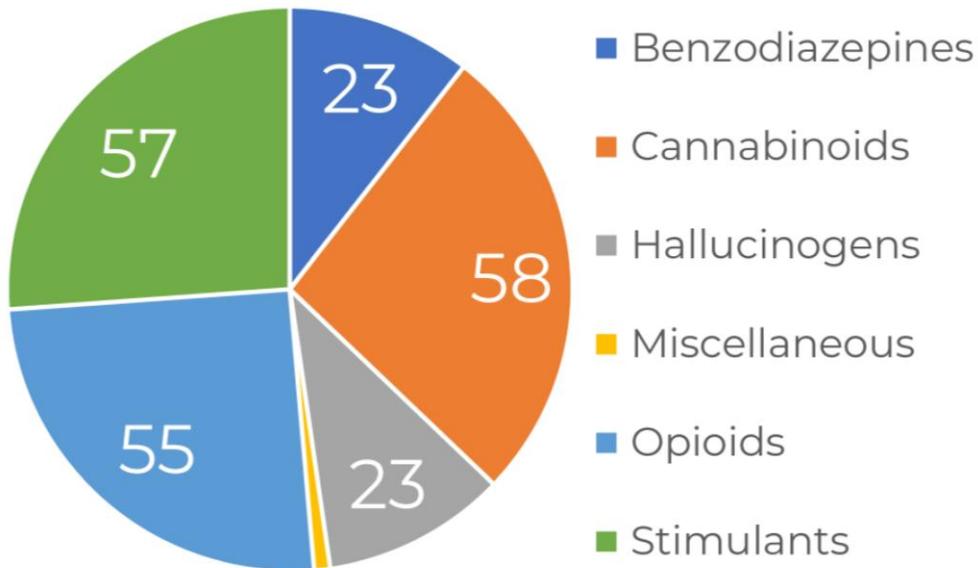


Figure 3: Breakdown by subclass of individual NPS detected, 2018-2022.

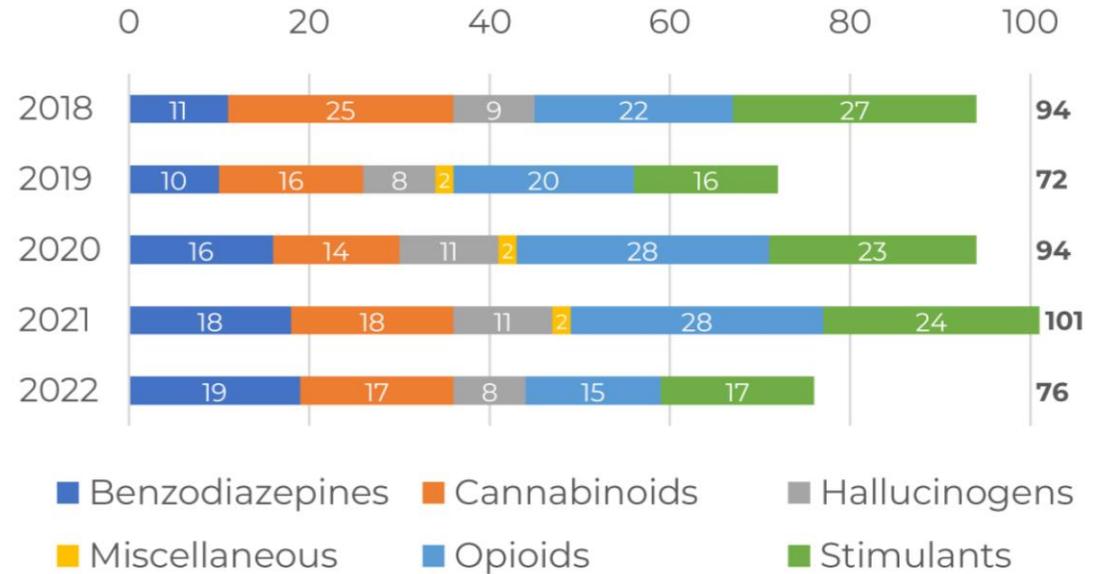


Figure 4: Individual NPS detected each year, cumulative since 2018.

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).

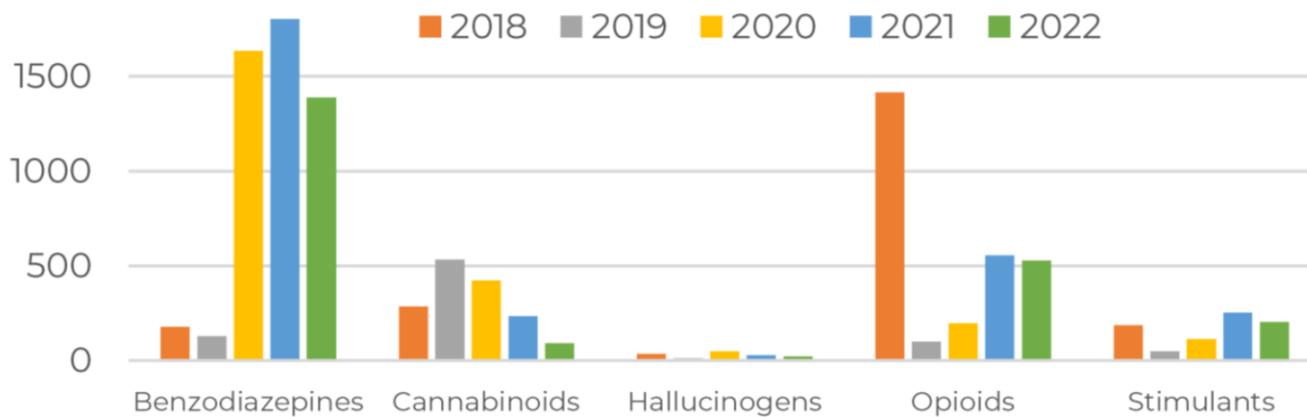


Figure 5: Total number of NPS detections among all samples analyzed since 2018.

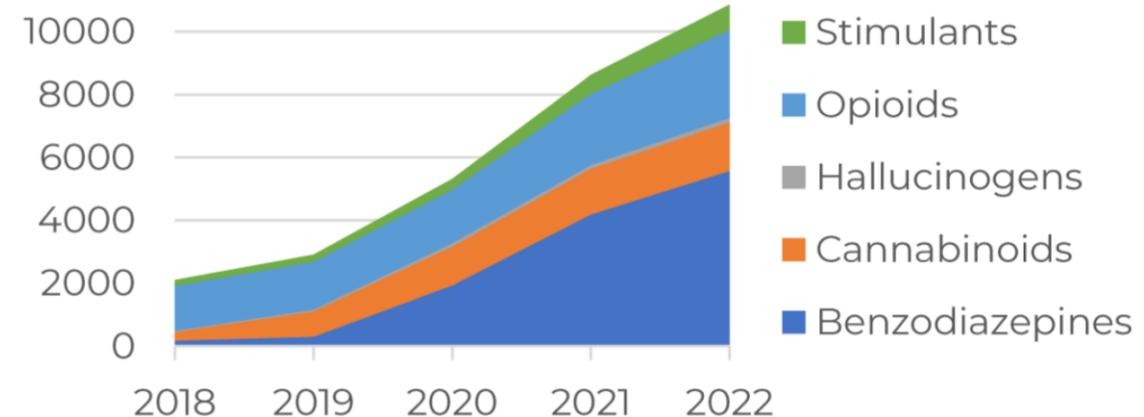
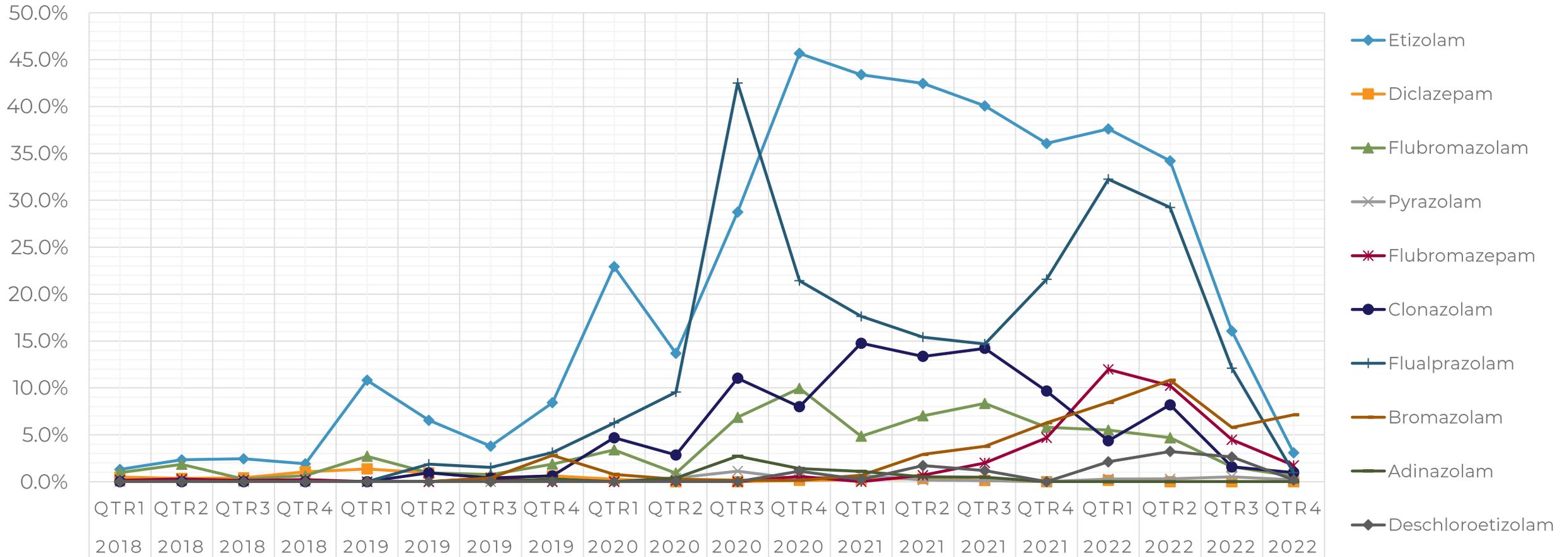


Figure 6: Cumulative number of NPS detections since 2018.

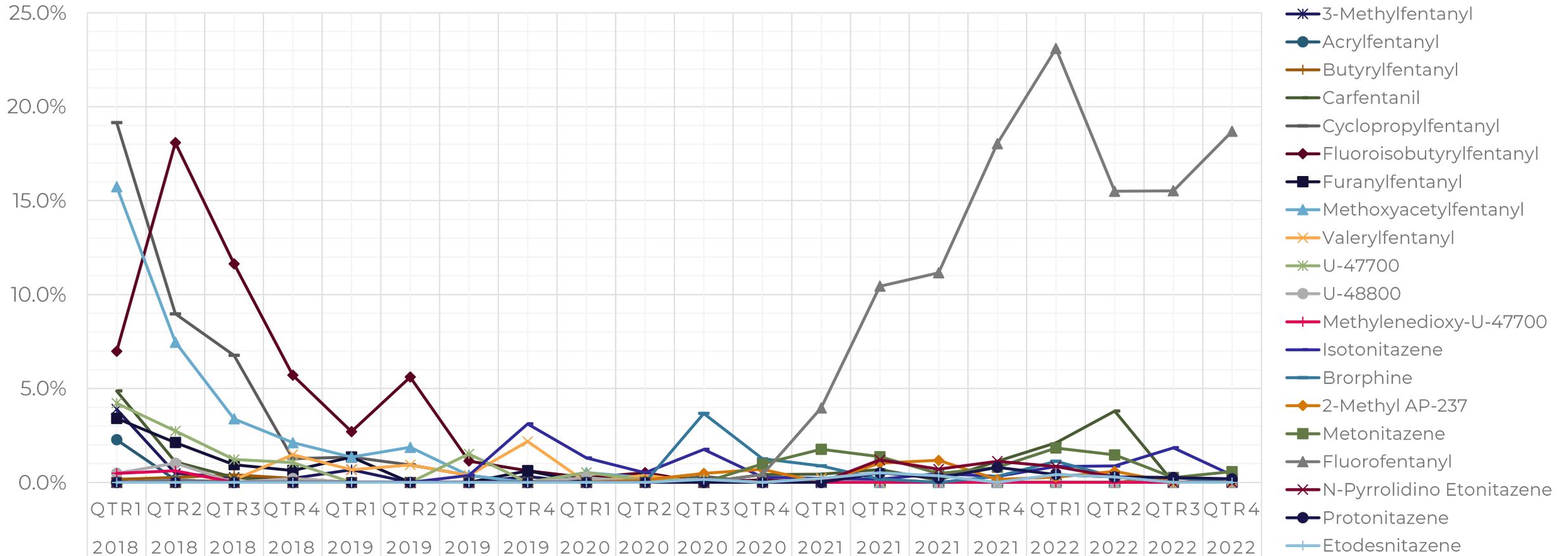
TRENDS FOR NPS SUBCLASSES IN THE U.S.

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



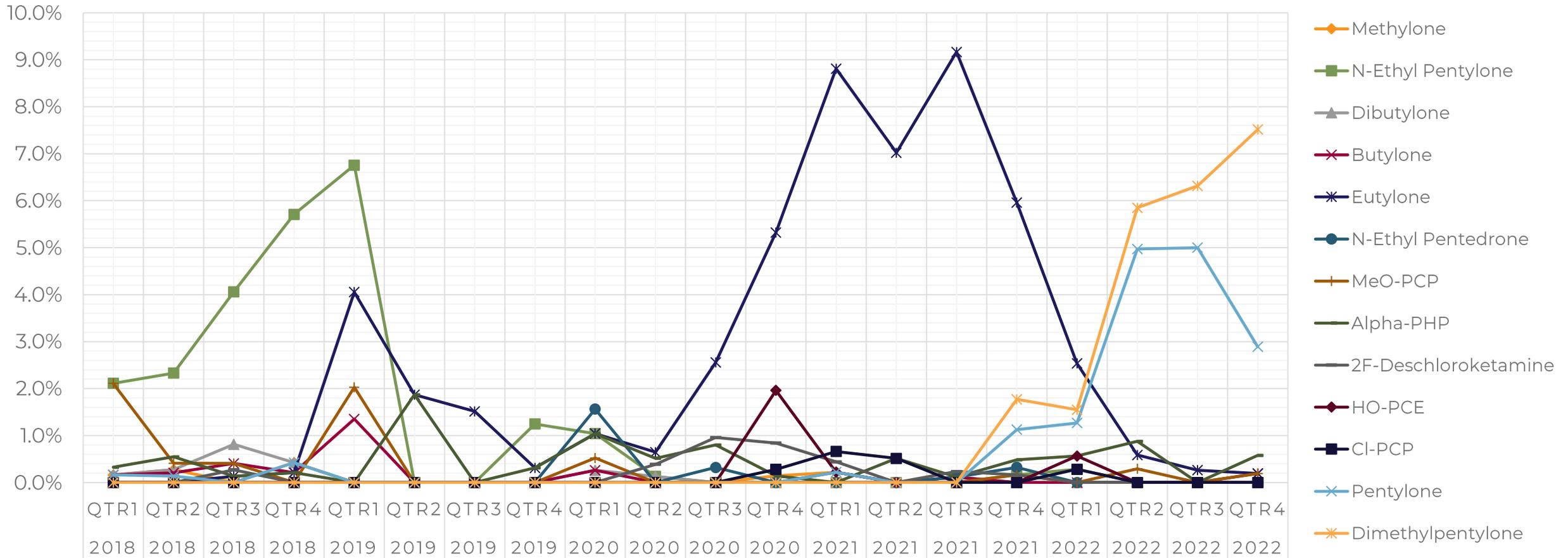
TRENDS FOR NPS SUBCLASSES IN THE U.S.

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



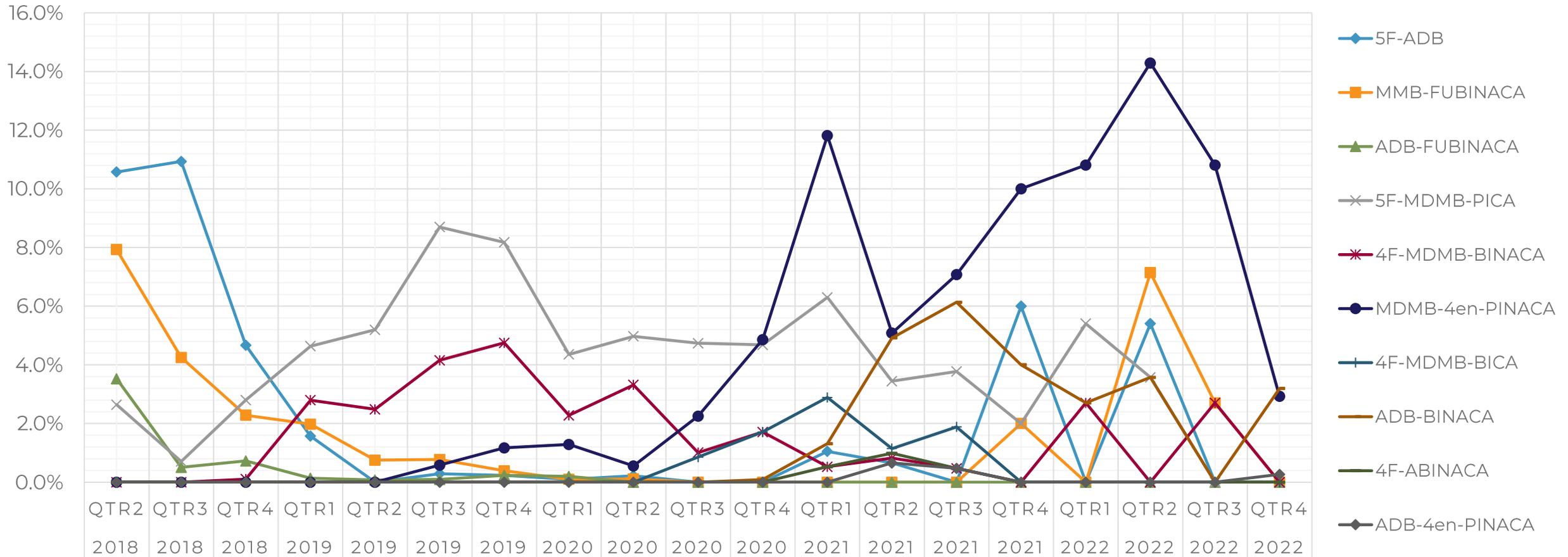
TRENDS FOR NPS SUBCLASSES IN THE U.S.

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



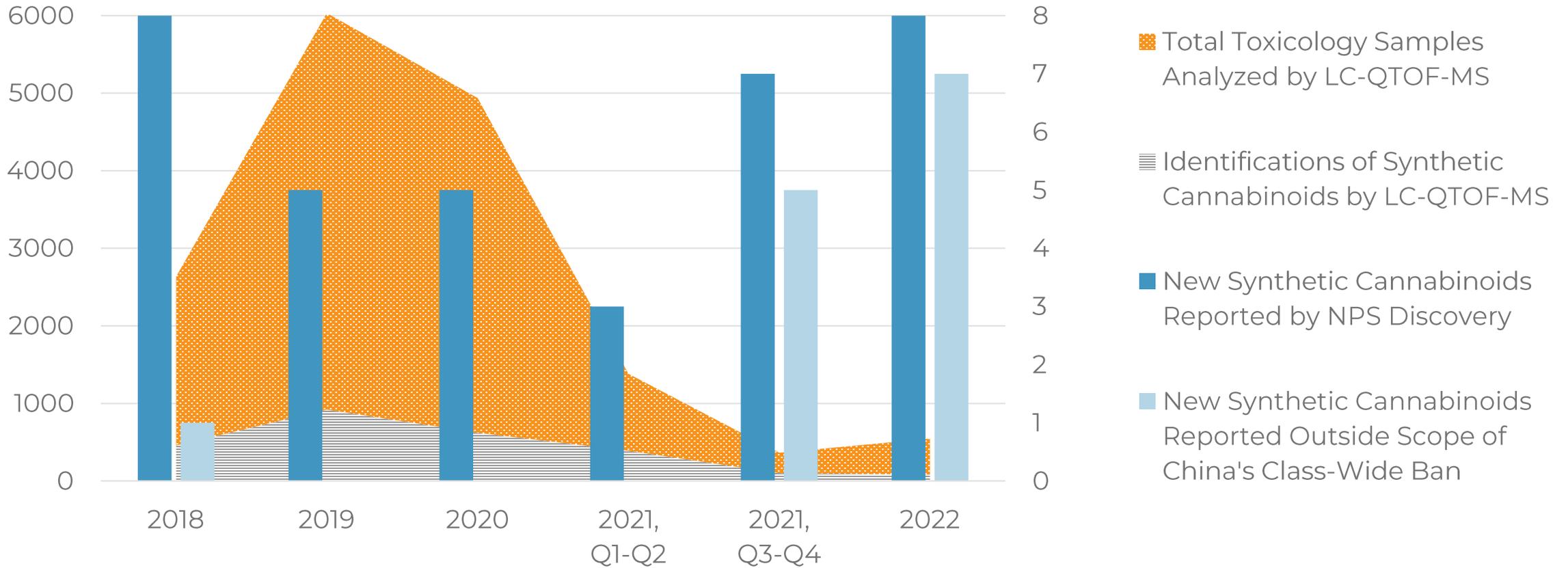
TRENDS FOR NPS SUBCLASSES IN THE U.S.

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



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 continued to increase since the mid-2000s, becoming the most frequently encountered opioid in the United States. Recreational fentanyl (isomers referred to as illicitly manufactured fentanyl) is the primary synthetic opioid identified in fatal drug overdoses, although there are increasingly reports of fentanyl poly-drug occurrences (e.g., in combination with xylazine, benzodiazepine, stimulants). Recreational fentanyl is commonly ingested through various routes of administration, including injection, smoking, and ingestion. Fentanyl remains a drug of high public health concern among an increasingly volatile drug supply, and its prevalence has thus far only increased despite various countermeasures.

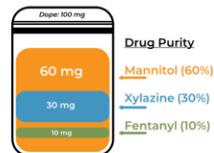


Figure 1: Illustration of drug purity.

WHAT ARE FENTANYL PRECURSORS, INTERMEDIATES, AND BYPRODUCTS?

Fentanyl is a synthetic drug produced via chemical processes and reactions between starting materials or precursors. Fentanyl can be chemically synthesized in different ways (Figures 5-9) using a variety of precursors. **Precursors** 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-ANP, benzofentanyl, phenethylchloride, and NPP. Examples of intermediates include 4-ANPP, 4-ANRP, and benzofentanyl. Examples of byproducts include 4-ANPP, phenethyl 4-ANPP, N-propionyl norfentanyl, and acetyl-fentanyl. To complicate matters, some molecules can be classified as a precursor and/or an intermediate and appear as a suspected byproduct (e.g., 4-ANPP) depending on the synthesis route. Based on currently available data or interpretive assessments, fentanyl precursors, intermediates, and byproducts are largely inactive or retain only very low opioid activity (with the exception of acetyl-fentanyl, making their presence in drug materials, especially in small quantities, of low pharmacological significance (although true toxicity of these chemicals remains unknown).

WHAT ARE PURITY AND POTENCY?

Purity is defined as the amount or quantity of a specific drug 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 10% (or 10 mg) for fentanyl, 30% (30 mg) for xylazine, 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 to 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 d-methamphetamine. Herein relating to fentanyl, purity is the amount of fentanyl in a product while the **Potency Index** (Figure 3) represents the combined effects of all opioids, including drugs like heroin and pure fentanyl. Potency index is calculated using relative pharmacological activity (EC₅₀), normalized, and expressed on a scale where a fentanyl powder of 10% purity 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 drug(s) 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 field 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 surges, 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).



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

Fentanyl Purity, Potency, & Synthesis (CONT.)

DRUG PURITY VS. POTENCY INDEX

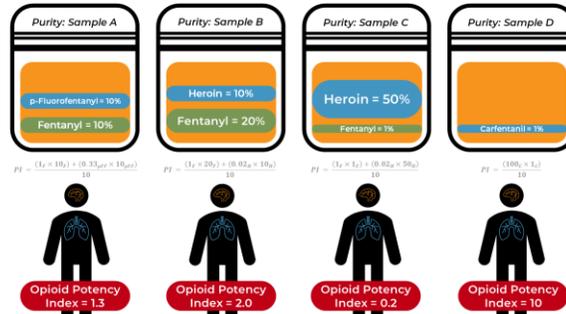


Figure 3: Illustration of drug purity (top) vs. potency index (bottom) with example calculations for opioids commonly encountered in the recreational drug supply.

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

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 $EC_{50}^{fentanyl} / EC_{50}^{opioid}$ at the mu opioid receptor.
- Examples of PFs: Fentanyl = 1, Heroin = 0.02, para-Fluorofentanyl = 0.33, ortho-Fluorofentanyl = 1, Carfentanyl = 10.
- Purity is the amount of drug in a specified sample and is expressed as a percent (e.g., 10%, 23%).
- [.] indicates that the numerator should be continued for all drugs (in this case opioids) present in the sample.
- The denominator is 10 – a Normalizing Factor applied to a sample of 10% fentanyl-only presents a Potency Index of 1.
- Potency index is reported to one decimal place (e.g., 0.9, 4.6, etc.) until the value eclipses 10.
- Tolerance, dose, and other use 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.

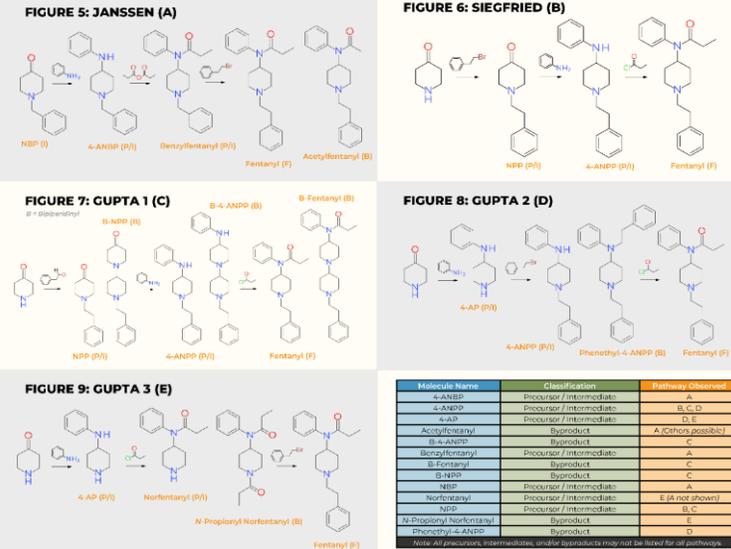


Fentanyl Purity, Potency, & Synthesis (CONT.)



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

FENTANYL SYNTHESIS PATHWAYS



Molecule Name	Classification	Pathway Observed
4-ANPP	Precursor / Intermediate	B, C, D
4-ANRP	Precursor / Intermediate	A
4-AP	Precursor / Intermediate	D, E
Acetyl-fentanyl	Byproduct	A (Others possible)
B-4-ANPP	Byproduct	A
Benzofentanyl	Precursor / Intermediate	A
B-Fentanyl	Byproduct	C
B-NPP	Byproduct	A
NPP	Precursor / Intermediate	A
Norfentanyl	Precursor / Intermediate	E (not shown)
N-Propionyl Norfentanyl	Precursor / Intermediate	B, C
N-Propionyl Norfentanyl	Byproduct	E
Phenethyl-4-ANPP	Byproduct	D

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

Disclaimer: In partnership between the Center for Forensic Science Research and...
Abbreviations: The authors acknowledge CFSRE and NPS for personnel for their...
Supporting Citation: ...

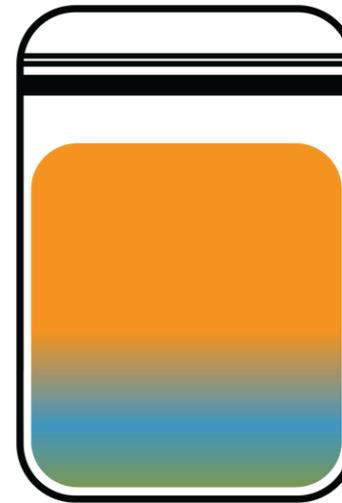
Author: ...
Supporting Citation: ...



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

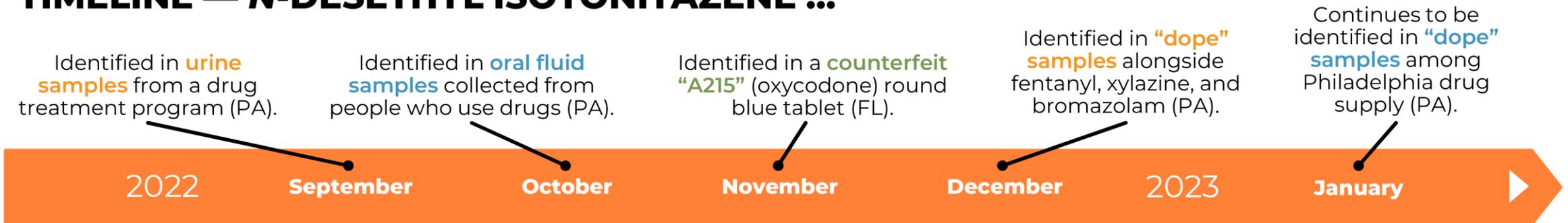
LOCATION: Philadelphia, PA, USA

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 ...





ACCESS NPS DISCOVERY & JOIN OUR LISTSERV

ACCESS NPS DISCOVERY REPORTS & JOIN OUR LISTSERV

NPS DISCOVERY

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 listserve](#) (be sure to select the NPS Discovery check box at the bottom):

Monographs | Trend Reports | Public Alerts

Intelligence | Drug Checking | Clinical Reports

Scope Recommendations | Analytical Toolkits | Podcasts & Media

History | Collaborators | Additional Content

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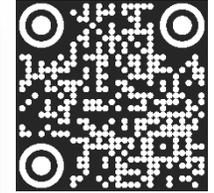
Student

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Questions?

alex.krotulski@cfsre.org

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