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The Center for Forensic Science Research & Education

NPS DISCOVERY

The Use of Combined Seized Drug and Toxicology Workflows for Rapid Identification of Emerging Substances in Response to Public Health Threats

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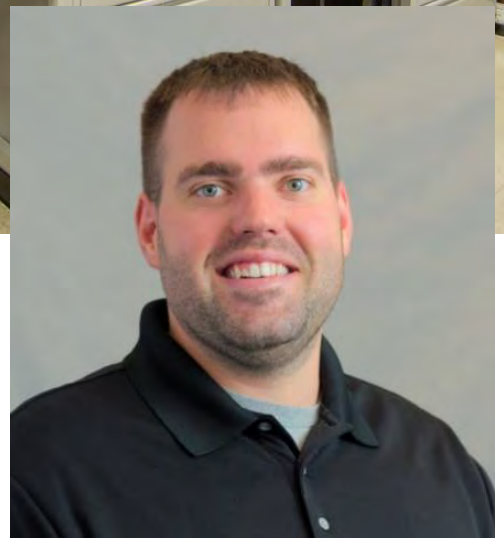
Workshop I — Identification, Characterization, and Analysis of NPS (Friday November 19, 2021)

The VIII International Conference on Novel Psychoactive Substances (NPS) – Virtual

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



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Disclosure and Introduction

- 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.
- I have worked in collaboration with:
  - NMS Labs – private toxicology laboratory
  - U.S. Customs and Border Protection
  - U.S. Department of Justice
  - Emergency Departments / Hospitals / Poison Control Centers
  - Medical Examiner and Coroner Offices / Forensic Laboratories

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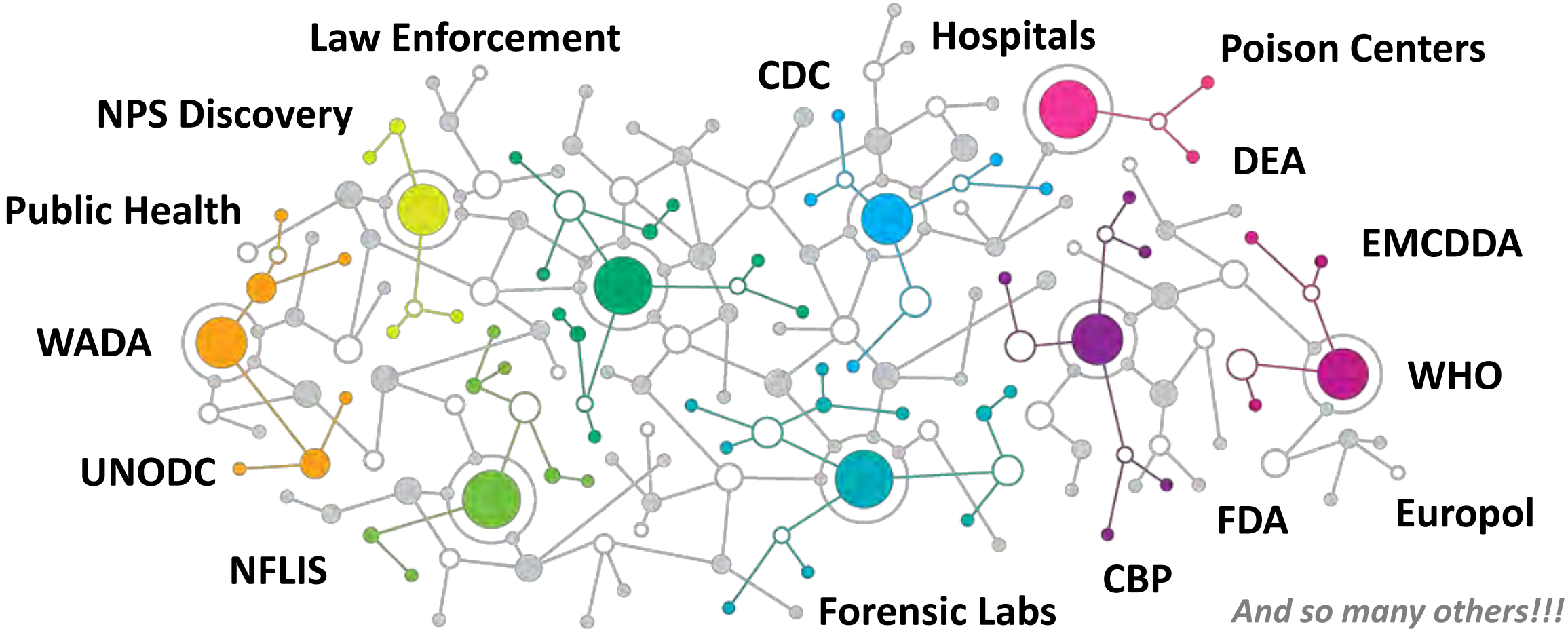
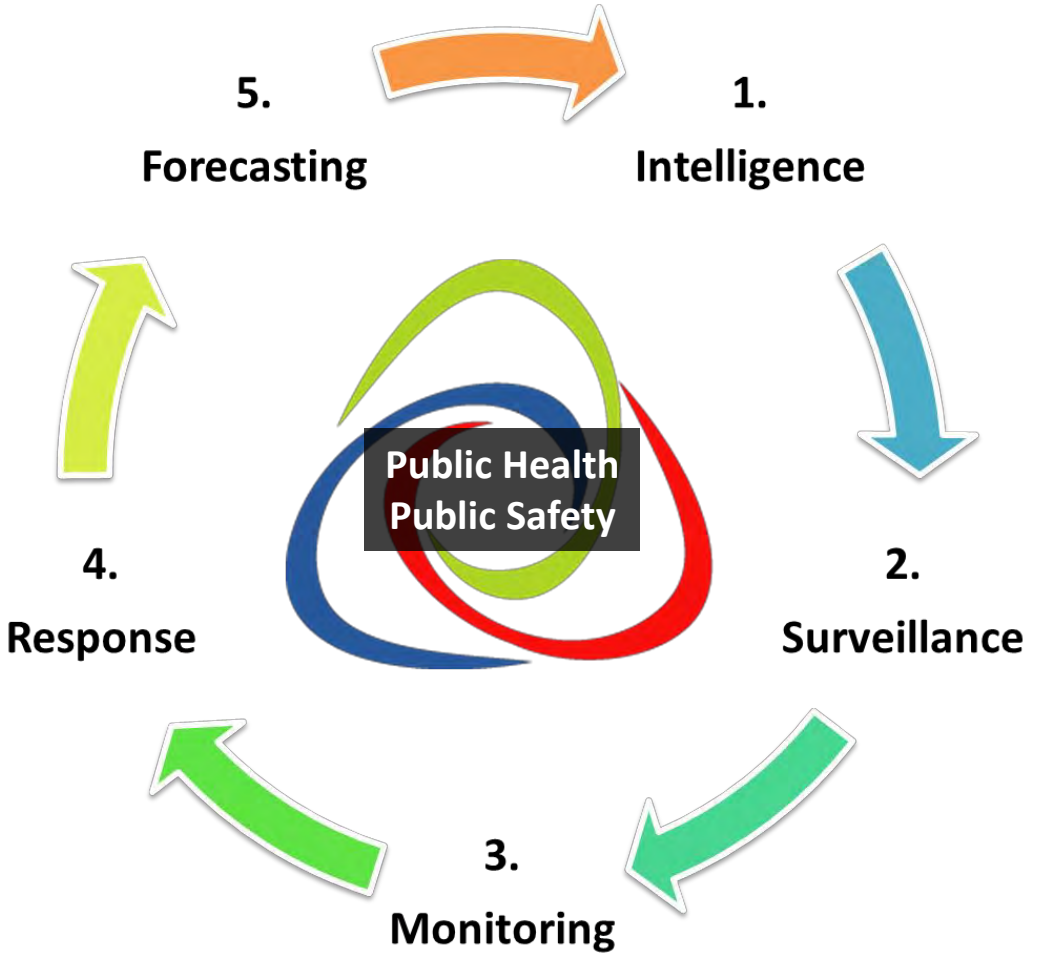
Drug Testing Network



NPS Discovery

- Open-access national drug early warning system in United States
- Launched in 2018
- Multidisciplinary program
- Focus on dissemination and outreach
- Intersection between:
  - Forensic Toxicology
  - Drug Chemistry
  - Clinical Intoxications



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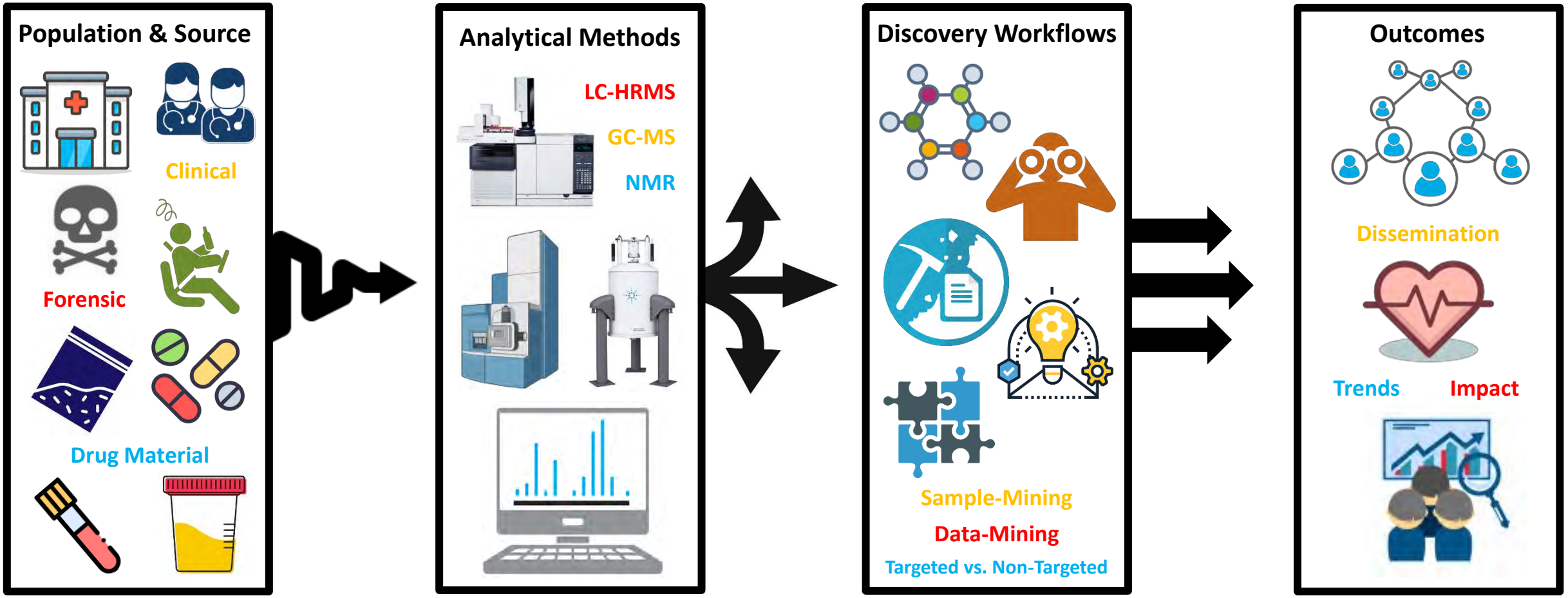
Presentation Overview

Our Process Overview



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SAMPLE SOURCE

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Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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ANALYICAL METHODS

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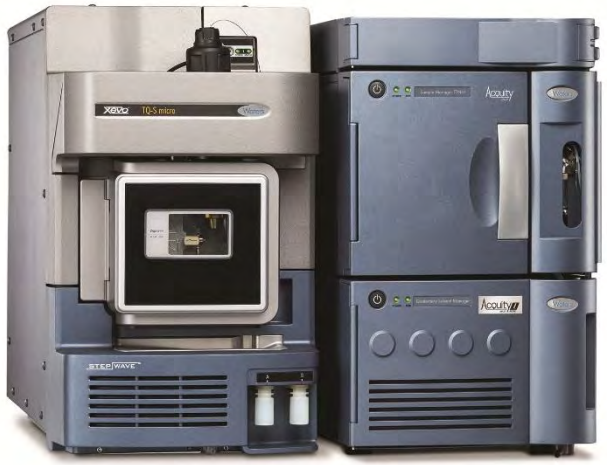
Non-Exclusive List of Instruments

- Immunoassays (EMIT, ELISA, etc.)
- **Mass spectrometry***
  - Gas chromatography mass spectrometry (GC-MS)
  - Liquid chromatography tandem mass spectrometry (LC-MS/MS)
  - High resolution mass spectrometry (HRMS)
    - Example: LC-TOF-MS, LC-QTOF-MS, LC-Orbitrap-MS
- Nuclear magnetic resonance (NMR) spectroscopy



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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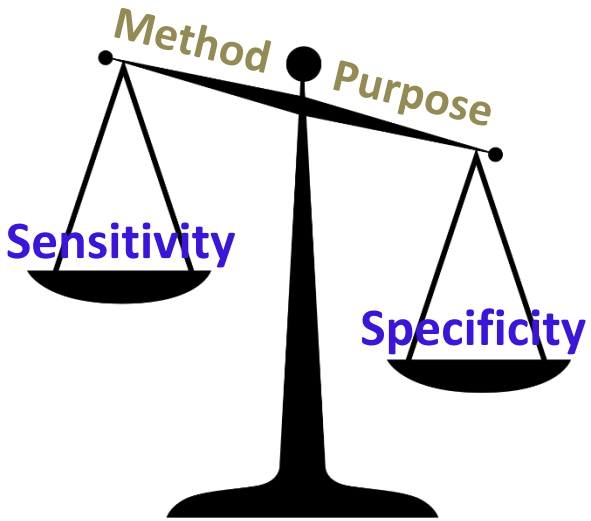
Sensitivity vs. Specificity

- **Sensitivity:**
  - Limits of detection
  - NPS concentration <1 ng/mL
- **Specificity:**
  - Differentiation power
  - NPS isomers
  - Retention time
  - Fragmentation pattern
- **Both dictate instrument to use and interpretation of results**
  - Run time / acquisition time
  - Sample throughput



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“Targeted” vs. “Non-Targeted”

- **Instrumental Analysis**
  - Data (or information) dependent acquisition (DDA or IDA)
    - Targeted analysis
    - Sample dictates outcome of results
      - “If x → then y” (mass)
  - Data independent acquisition (DIA)
    - Non-targeted analysis
    - Outcome (acquisition) regardless

- **Data Processing**
  - Targeted
    - Defined scope of testing
    - Library/database (mass list, XIC list)
    - Processing criteria
  - Non-targeted
    - “Suspect screening”…*
    - Unknown searching
    - Difficult and time consuming

- **… Interpretation / Discovery**

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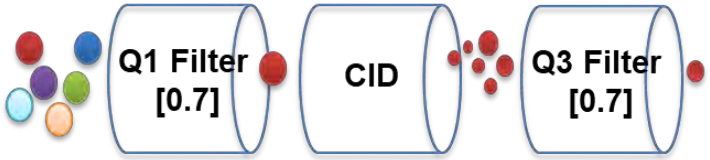
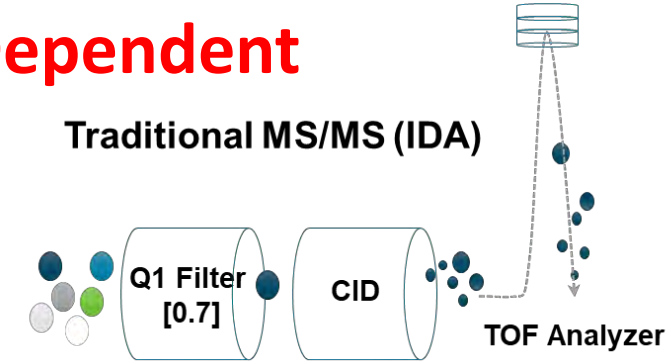
“Targeted” vs. “Non-Targeted”

Targeted / Data Dependent

Traditional “Triple Quad”



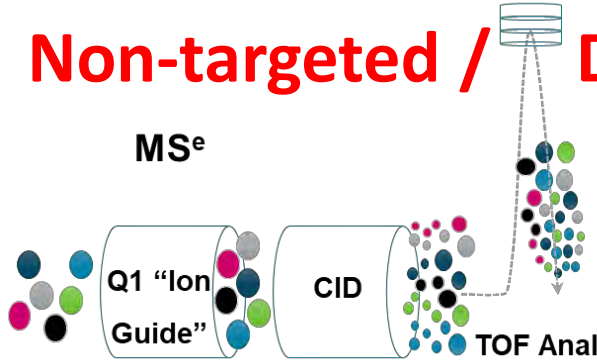
Traditional MS/MS (IDA)



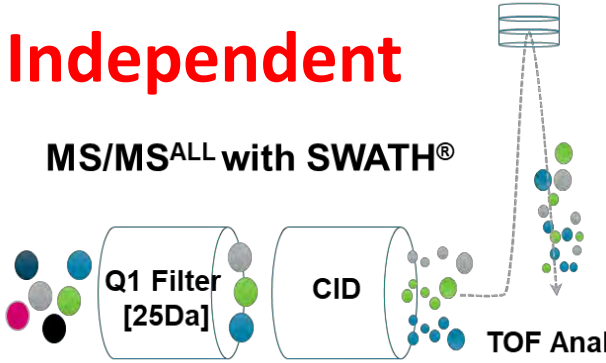
Non-targeted / Data Independent

“Single Quad” / Scan

MS^{e}



MS/MS^{ALL} with SWATH®



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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Instruments and Utility

- **Screening**
  - Low specificity
  - Low accuracy
  - GC-MS*
  - LC-TOF-MS
  - Drug discovery
  - “Non-targeted”
- **Identification**
  - Moderate specificity
  - High accuracy
  - GC-MS*
  - LC-QTOF-MS
  - Drug discovery
  - Drug characterization
  - “Targeted/Non-targeted”
- **Confirmation**
  - High specificity
  - High accuracy
  - LC-MS/MS
  - NMR
  - Drug characterization
  - Absolute identify
  - Quantitation
  - “Targeted”

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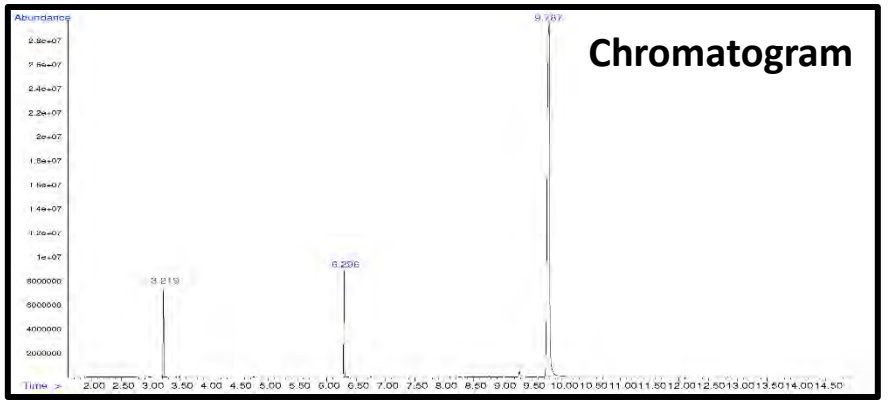
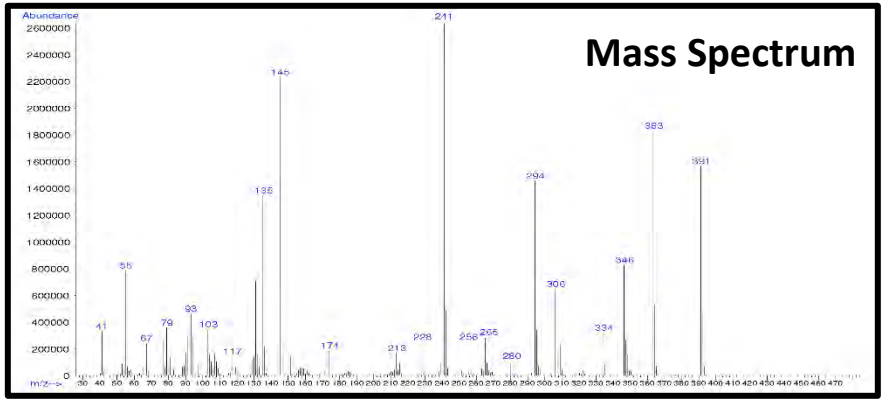
GC-MS Method

- **Sample Preparation:**
  - Drug samples – acid/base extraction
- **Instrument:**
  - Agilent 5975 Series GC/MSD →
- **Column & Carrier Gas:**
  - Zebron™ Inferno™ ZB-35HT
  - Helium (Flow: 1 mL/min)
  - Temperature program
- **MS Parameters:** 40-550 m/z





Sample Source	Analytical Methods
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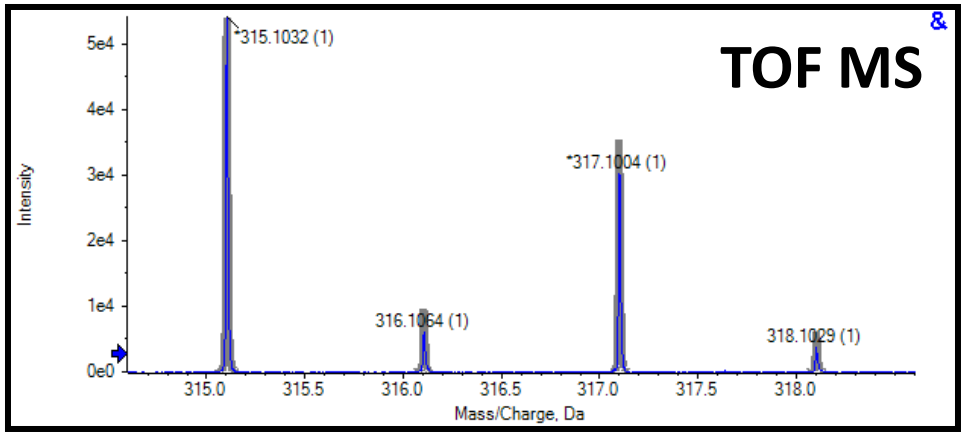
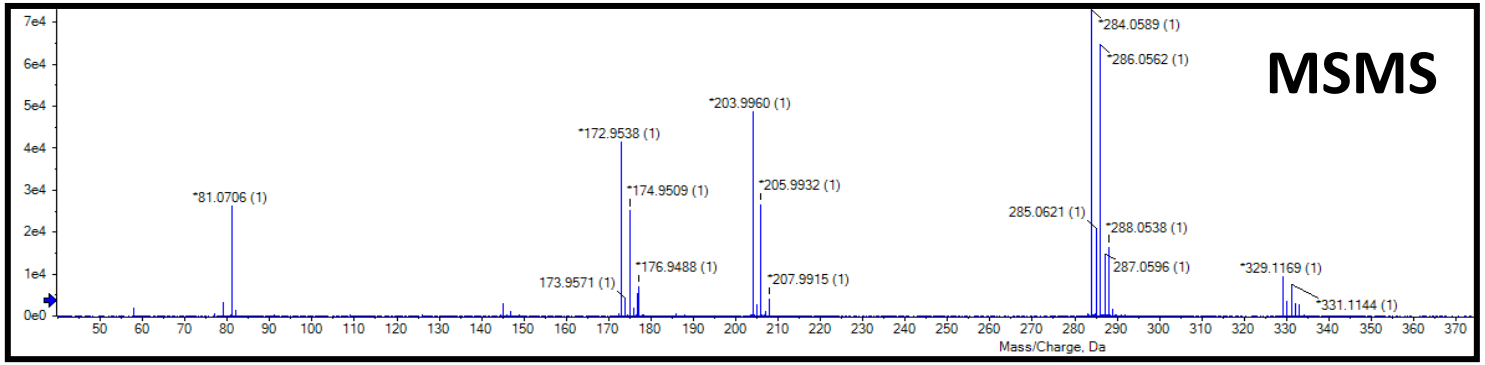
General LC-QTOF-MS Parameters

- **Sample Preparation:**
  - 1:100 dilution of GC-MS extract
  - Extraction of biological samples
- **Instrument:**
  - Sciex TripleTOF® 5600+ →
- **Column and Mobile Phase:**
  - Phenomenex® Kinetex C18
  - A: Ammonium formate (10 mM, pH 3.0)
  - B: Methanol/acetonitrile (50:50)
- **MS Parameters:** 100-510 Da





Sample Source	Analytical Methods
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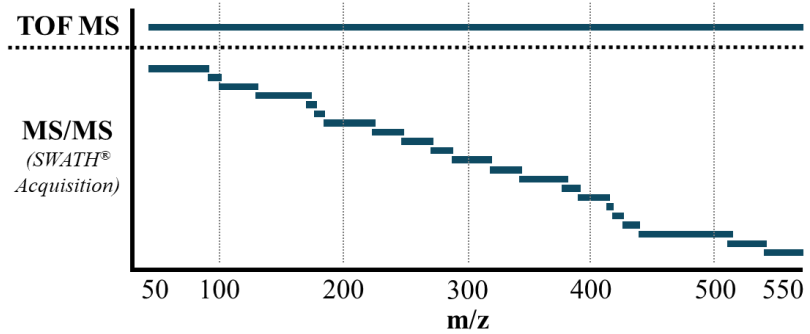
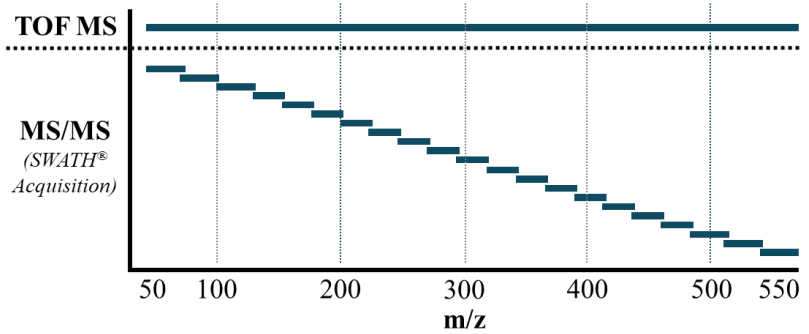
Two LC-QTOF-MS Methods

- **Basic Drug Method**
  - Generic LC gradient
  - SWATH® Acquisition



  - 15.5-minute method
  - **800+ drugs of abuse, NPS, metabolites, etc.**

- **Synthetic Cannabinoid Method**
  - Generic LC gradient
  - SWATH® Acquisition



  - 7-minute method
  - **250+ synthetic cannabinoids (parent and metabolites)**

Sample Source	Analytical Methods
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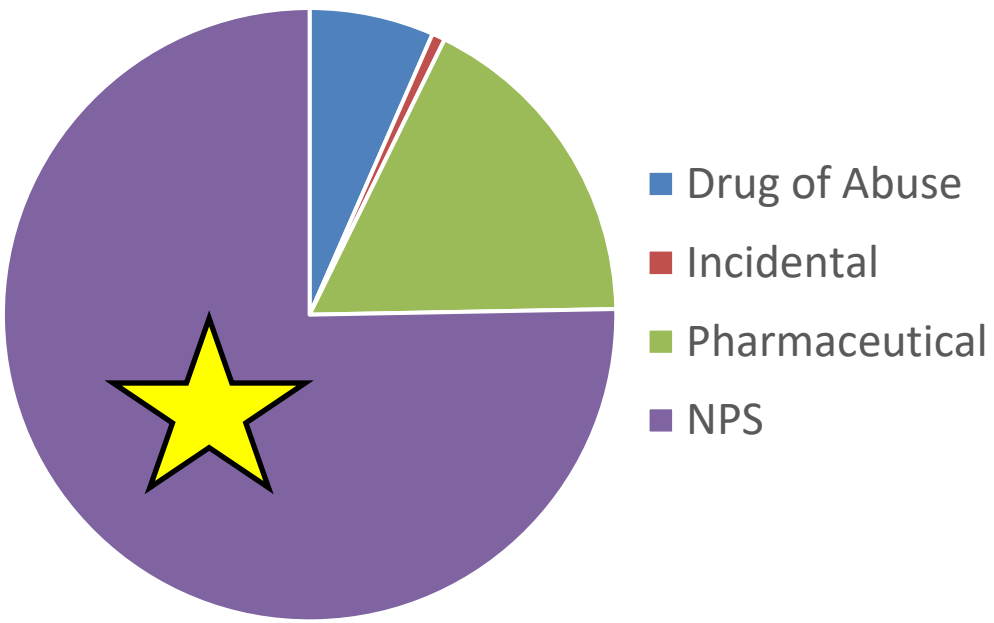
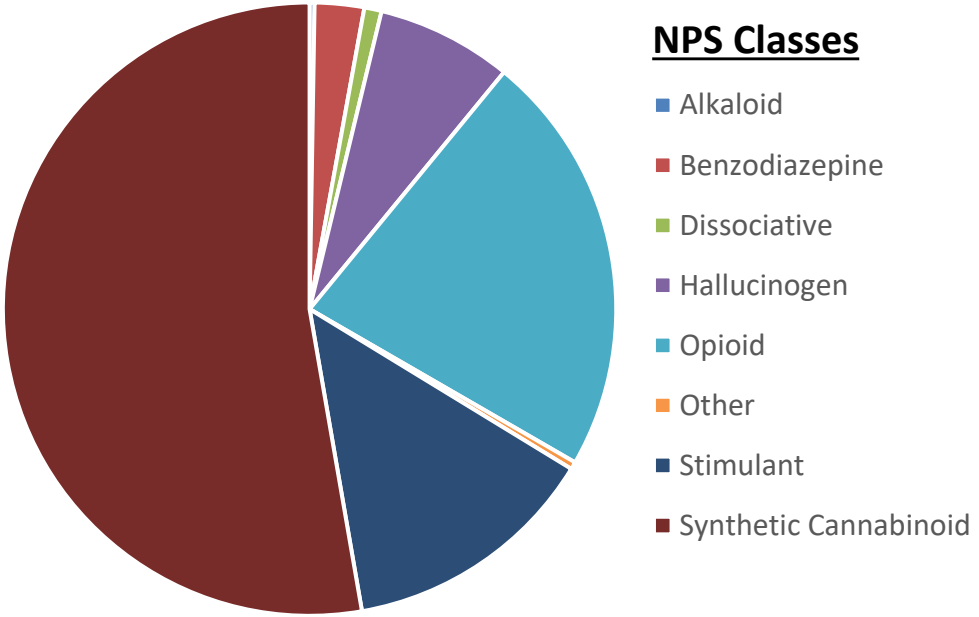
Standards / Library Database

- >950 standards in library database





HRMS Identification Criteria

- Criteria should be consistent across industries
- Setpoints can be related to certainty of the method (screen vs. identification)
- Evaluated based on experimental data

- Strict vs. lenient
- Manual vs. automated

	Mass Error Mass Error (ppm)	**Retention Time** Delta (min)	**Isotope** Isotope Ratio % Difference	**Library Hit** Library Score
✓	< 5.0	< 0.25	< 30.0	> 90.0
▲	< 10.0	< 0.35	< 50.0	> 70.0
●	>= 10.0	>= 0.35	>= 50.0	<= 70.0

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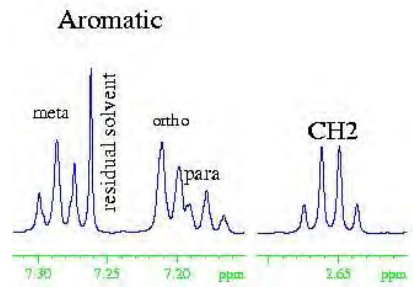
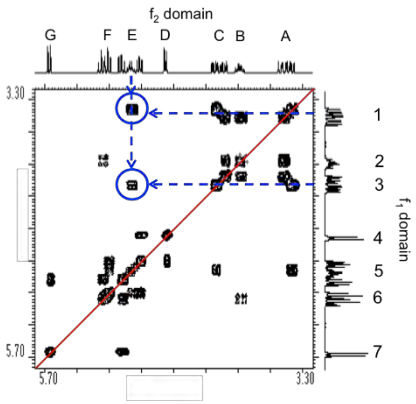
NMR Method

- **Sample Preparation:**
  - Dilute powder in CDCl3
- **Instrument:**
  - 300 MHz INOVA VARIAN Spectrometer →
- **Pulse Sequence:** Proton
- **Spectral Width:** 4798.5 Hz for 1D (-2 – 14 ppm) and 3773.6 for 2D
- **Delay between pulses:** 1st delay, d1 = 1.000





Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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Example LC-MS/MS Method

- **Sample Preparation:**
  - Extraction of biological samples
- **Instrument:**
  - Waters Xevo TQ-S micro →

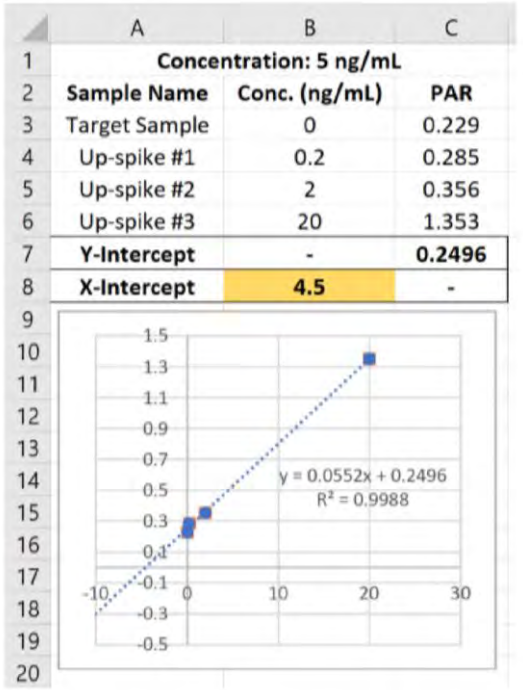
- **Column and Mobile Phase:**
  - Agilent InfinityLab Poroshell 120 EC-C18
  - (3.0 x 100 mm, 2.7 µm)
  - A: 0.1% formic acid in water
  - B: 0.1% formic acid in methanol
- **MS Parameters:** MRM
- **Quantitation:** Standard Addition

Table I. LC Gradient Conditions

Time (min)	%A	%B	Flow (mL/min)
Initial	50	50	0.4
1.0	50	50	0.4
4.0	5	95	0.4
5.0	5	95	0.4
5.1	50	50	0.4
6.0	50	50	0.4

Table II. MRM Parameters

Analyte	Cone (V)	Precursor (m/z)	Collision (V)	Product (m/z)	Dwell (s)
Isotonitazene	50	411.2	46	106.9	0.053
			22	100.0	0.053
			44	72.0	0.053
Fentanyl-d_5	56	342.2	24	188.0	0.053
			40	105.0	0.053



	A	B	C
1	Concentration: 5 ng/mL		
2	Sample Name	Conc. (ng/mL)	PAR
3	Target Sample	0	0.229
4	Up-spike #1	0.2	0.285
5	Up-spike #2	2	0.356
6	Up-spike #3	20	1.353
7	Y-Intercept	-	0.2496
8	X-Intercept	4.5	-

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DISCOVERY WORKFLOWS

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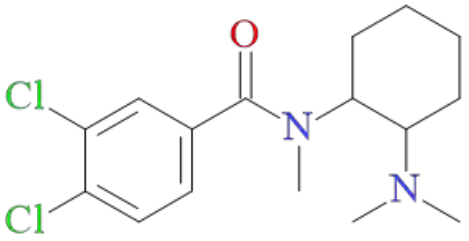
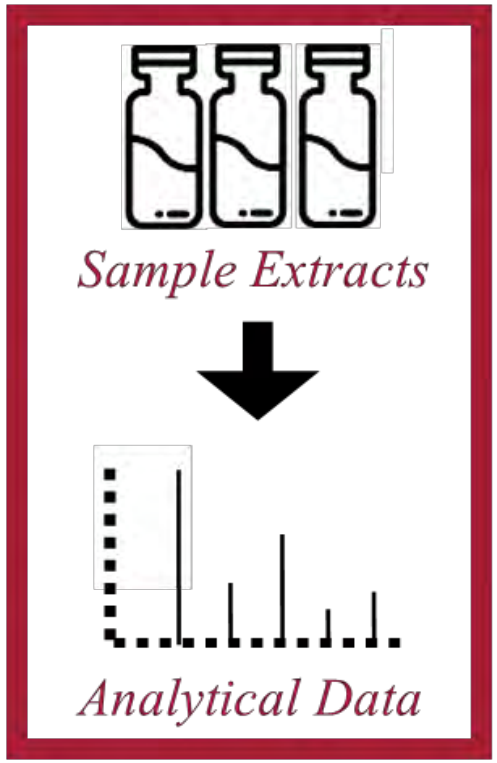
Sample-Mining vs. Data-Mining

PAST:
Retrospective

Data Mining

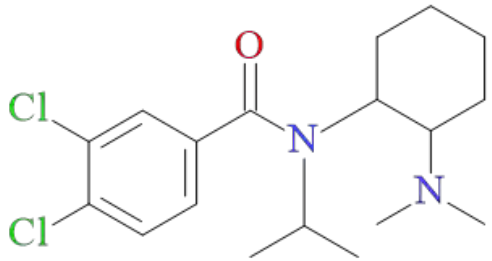


When was U-47700 first detected but not identified?



PRESENT:
Real-Time

Sample Mining



When will isopropyl-U-47700 first be detected and identified?

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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Sample-Mining vs. Data-Mining

- **Considerations for successful SM and DM:**
  - Scope of testing → Differs from standard laboratory scope of testing
  - Comprehensive in nature – not limited by drug class (but, limited by sample preparation)
  - Consistency in methods and sample acquisitions, especially for data-mining
  - Need software or application to assist with data processing and storage

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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Sample-Mining

- Sample Receipt
- Preparation/Extraction
- Analysis
- Data Processing
- Interpretation*
- Reporting

Discarded Extracts

NMS LABS

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Database (>950 drugs)

NPS

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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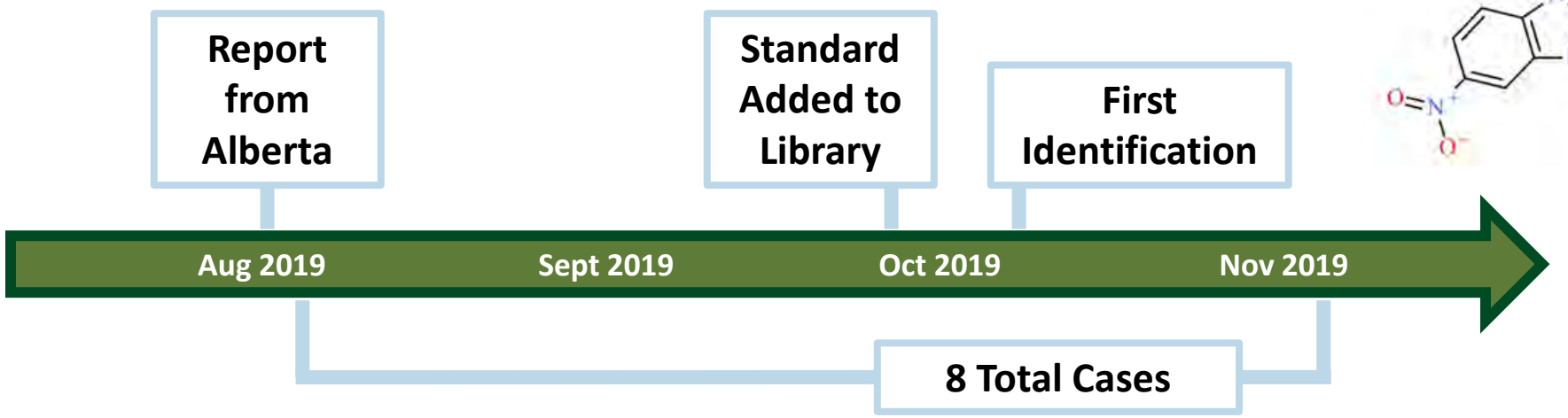
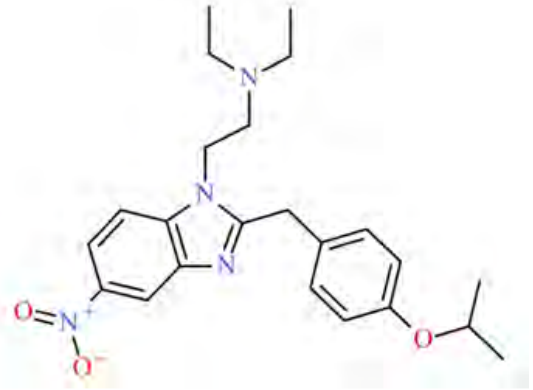
Data-Mining Example: Isotonitazene





Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Toxicologically relevant identifications that were "missed" during initial data processing

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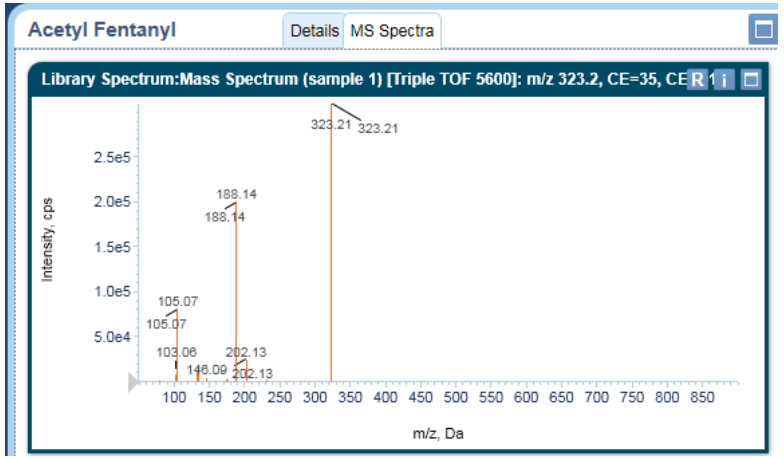
Library Databases

- Three main types:
  - In-house
  - Commercial
  - Publicly available
- Used for:
  - Targeted data processing
  - "Suspect screening"

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

- Information included:
  - Analyte name
  - Formula → exact mass
  - Retention time
  - Fragmentation pattern
- ***Results are only as good as their identifications…***

Name	Extraction Mass (Da)	Expected RT (min)	Fragment Mass (Da)
Alpha-PVP	232.16959	5.1	
Alpha-PVP	232.16959	5.1	232.1703
Alpha-PVP	232.16959	5.1	91.0556
Alpha-PVP	232.16959	5.1	126.1281
Alpha-PVP	232.16959	5.1	105.0344
Alpha-PVP	232.16959	5.1	161.0958



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Additional Lab Discovery Workflows

- **In-Scope Findings vs. Out-of-Scope Findings (OSF) at NMS Labs**
  - In-scope findings correlate to the laboratories standard scope of testing
  - Out-of-scope findings are additional drugs above and beyond the standard scope
    - Tier 1: Confirmation methods available
    - Tier 2: Intelligence gathering purposes

- **Added MRM Transitions to LC-MS/MS Methods (OCME's)**
  - Laboratory may have several different confirmations panels
  - Based on intelligence, the lab might add suspected or confirmed precursor-product ion transitions to methods
  - "Background drug monitoring" helps drive future testing development
    - Reportable results???

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OUTCOMES & SUCCESS STORIES

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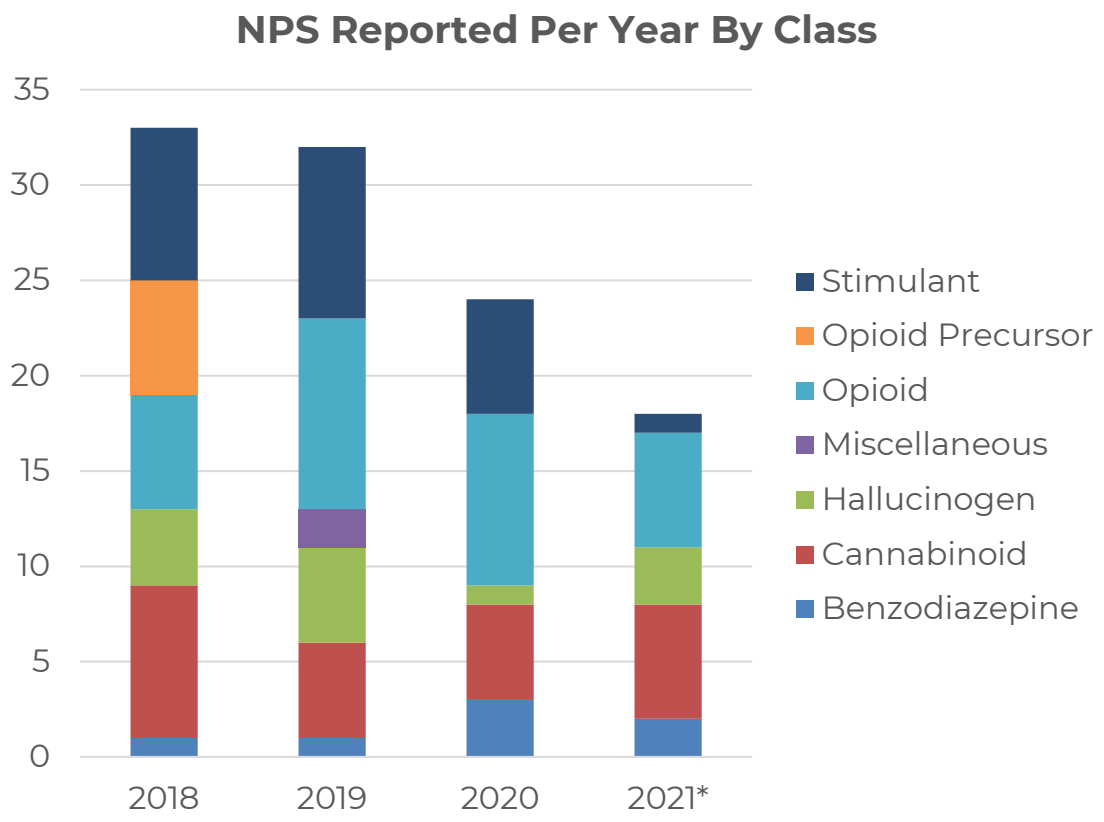
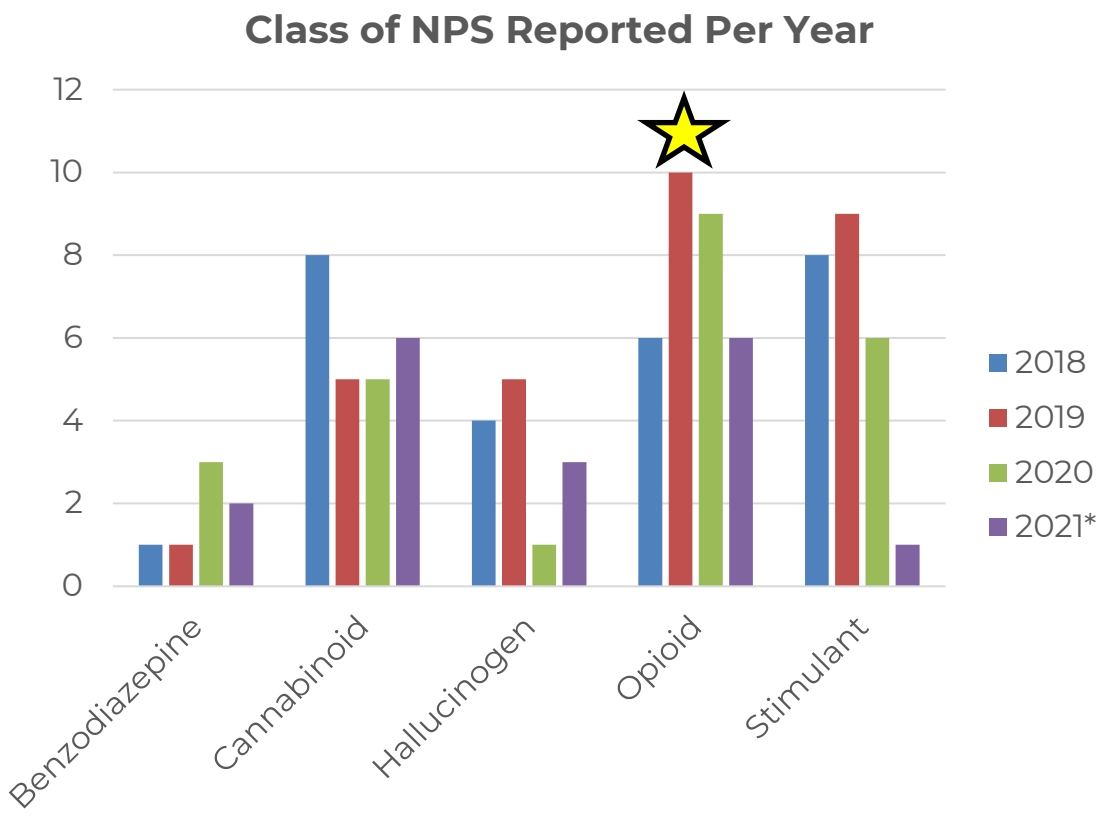
Discovery of New NPS





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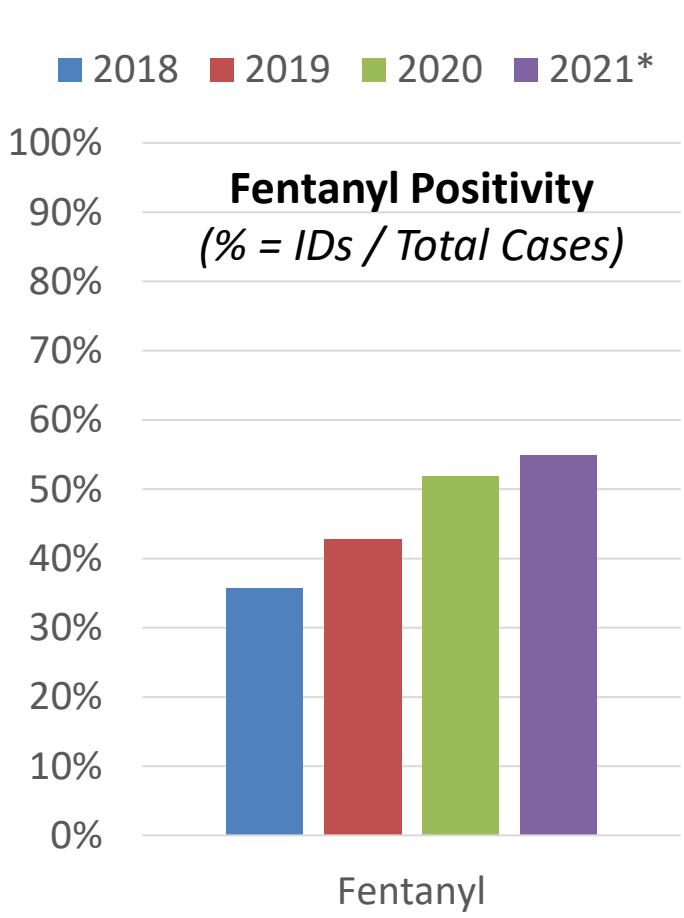
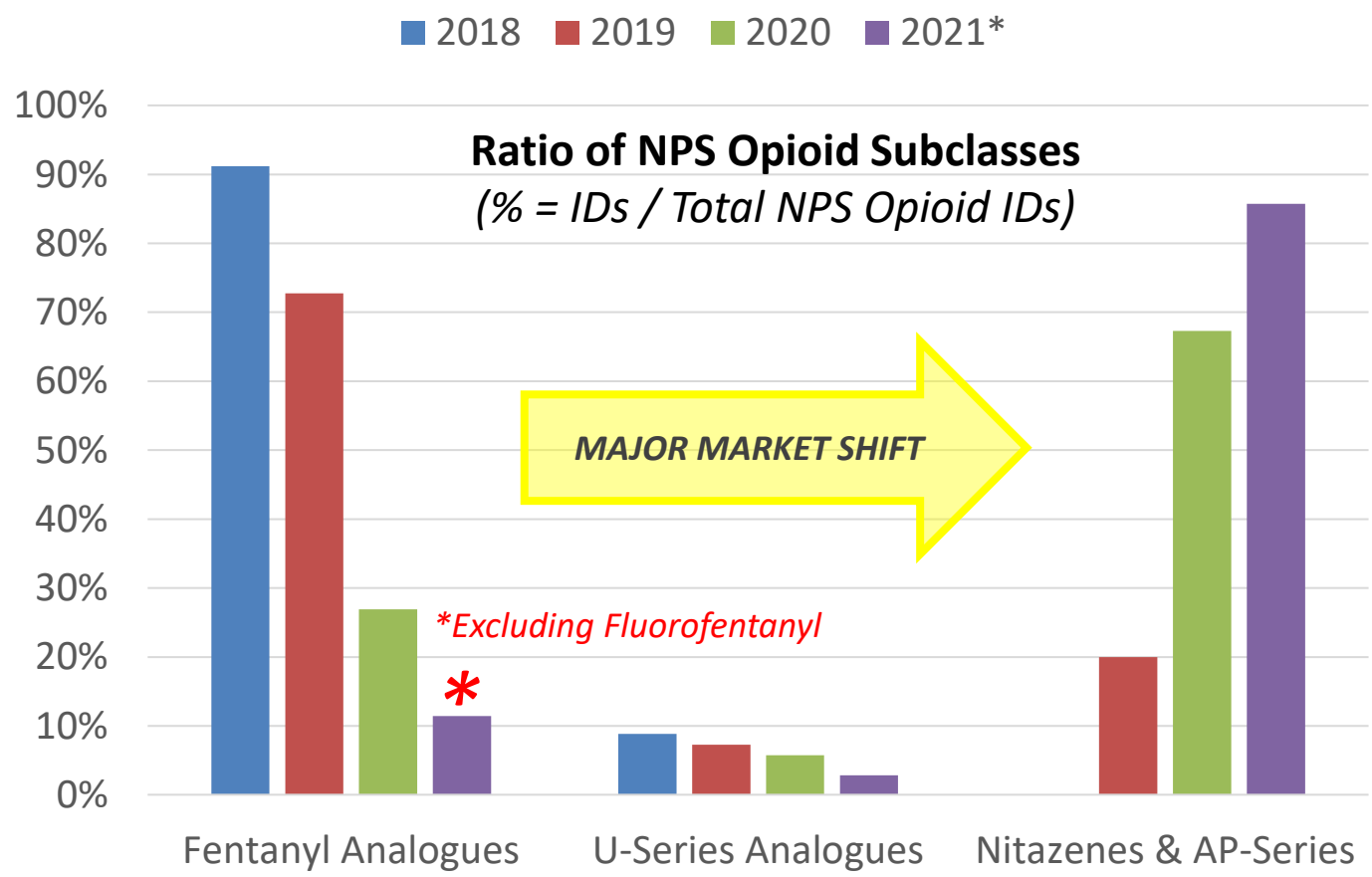
Positivity of NPS Opioids





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“Santa Muerte” Outbreak in Philadelphia, PA

- Circumstances:
  - Late July weekend in Philadelphia (2018)
  - >160 overdoes and >10 deaths
  - “Santa Muete” stamped heroin bags →
  - Believe to be the last of the “pure” heroin
  - EMS responded and several local hospitals were admitting patients
  - Patients were administered naloxone, but became agitated and confused
  - Scopolamine/anticholinergic toxidrome?

- CFSRE received seized “heroin” powder and biological samples
  - Testing by GC-MS and/or LC-QTOF-MS

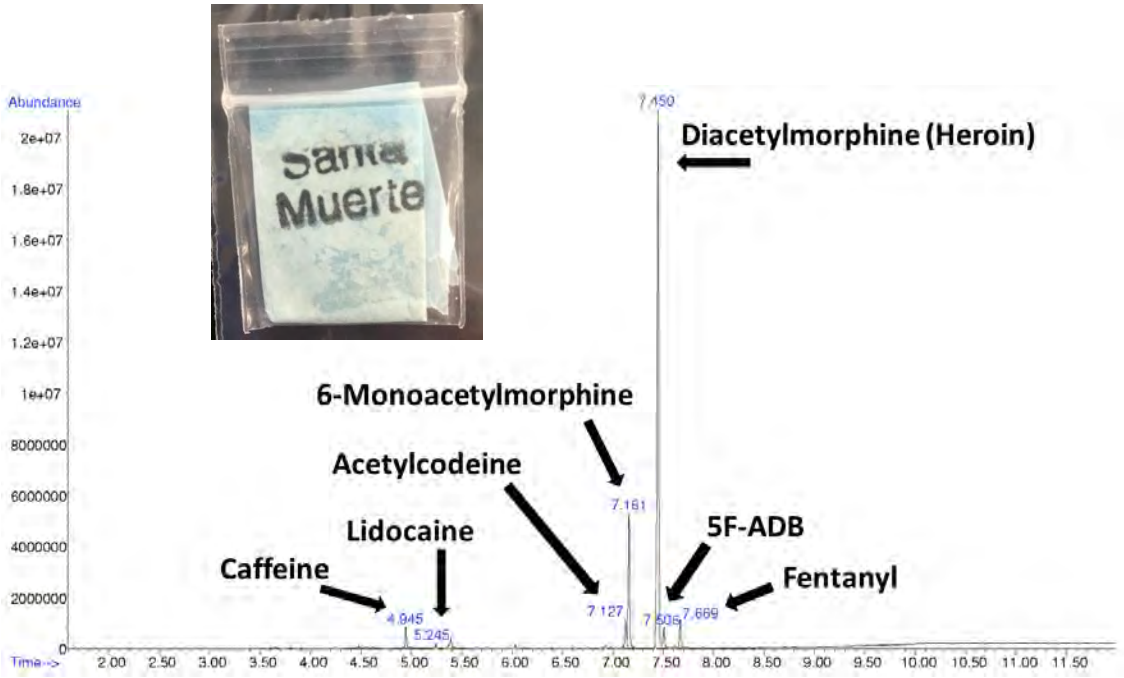
Philly overdose drug may have contained toxic designer drug

by Aubrey Whelan and Mari A. Schaefer, Updated: July 26, 2018

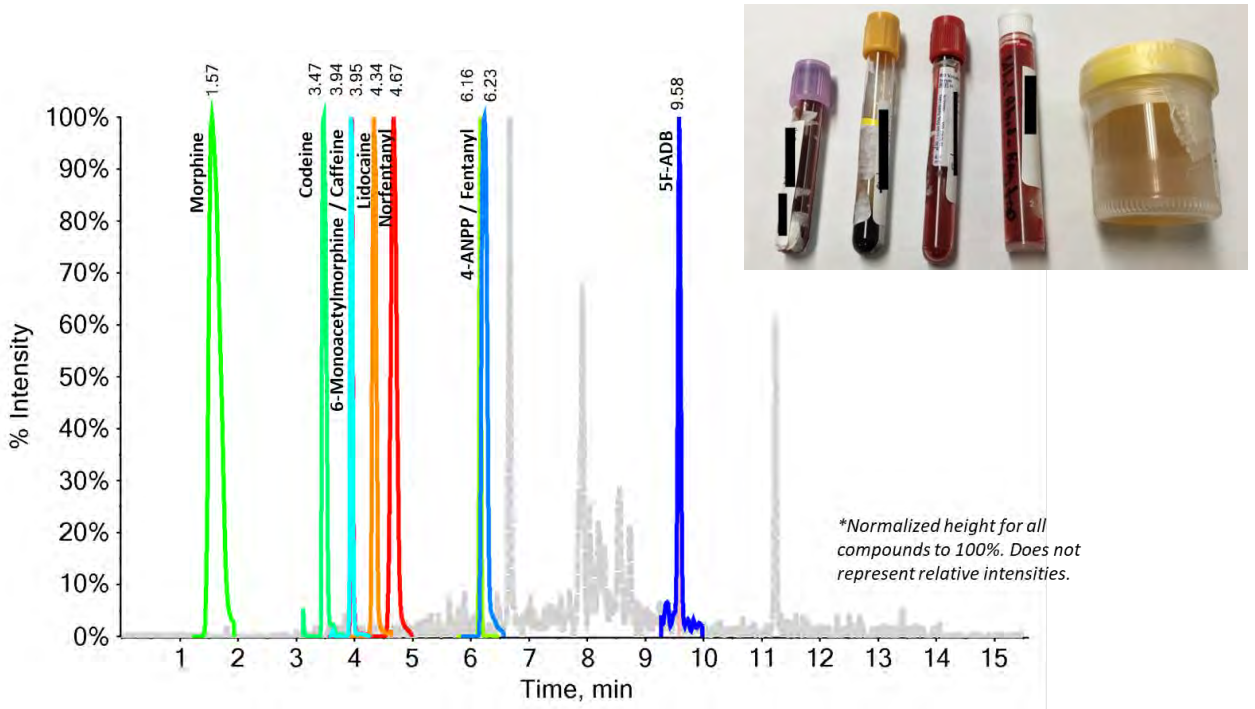
DAVID MAIALETTI

“Santa Muerte” Outbreak in Philadelphia, PA

- Seized drug sample



- Biological samples



***First time finding synthetic cannabinoids with heroin/fentanyl. Changes to future treatment/administration of naloxone?**

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Partnerships with Clinicians & Hospitals

- Patients present to ED after overdose
- Clinician suspect synthetic drug use
  - Outside hospital scope of testing
- Waste/residual biological samples
  - Sent to CFSRE
- Analyzed via LC-QTOF-MS

- Results reported back within ~2 weeks; Clinicians can adjust future treatment, etc.

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Partnerships with Clinicians & Hospitals

- **Case 1:**
  - 63 y/o male behaving erratically in public
  - Tachycardic, hypertensive, and hyperthermic
  - Required multiple doses of antipsychotics and benzodiazepines to sedate
  - Acute kidney injury and rhabdomyolysis
  - Admitted to taking “everything under the sun” on a multi-day binge
  - Urine drug screen was positive for amphetamines and opiates
  - Toxidrome was most consistent with sympathomimetics or synthetic cannabinoids
  - **Result: 4F-MDMB-BICA (cannabinoid)**
- **Case 2:**
  - 31 y/o found unresponsive at home
  - Respiratory depression
  - EMS administered naloxone
  - Transported to hospital
  - Vitals: HR 65, BP 122/71, RR 18, 97.9°F
  - 24 later, opioid toxidrome resolved
  - Hospital drug screens negative for opioids
  - Patient admitted to taking “2-methyl AP-237” which he purchases and mixed with water
  - **Results: 2-Methyl AP-237 (opioid) and concomitant use of NPS benzodiazepines**

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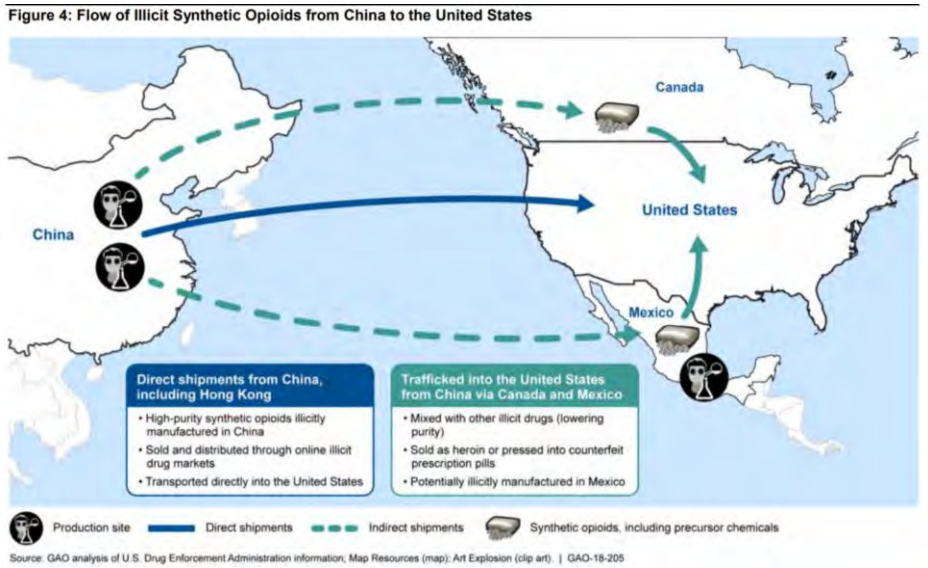
New Drugs Entering the United States

- Unidentified powders
- Agents suspect synthetic drugs present
- Powders sent to CFSRE
- Analyzed via GC-MS, LC-QTOF-MS, & NMR
- Correlation to street drugs supply and toxicology samples



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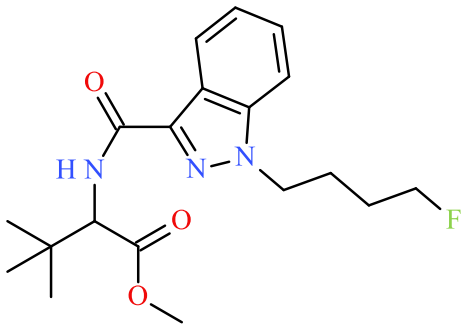
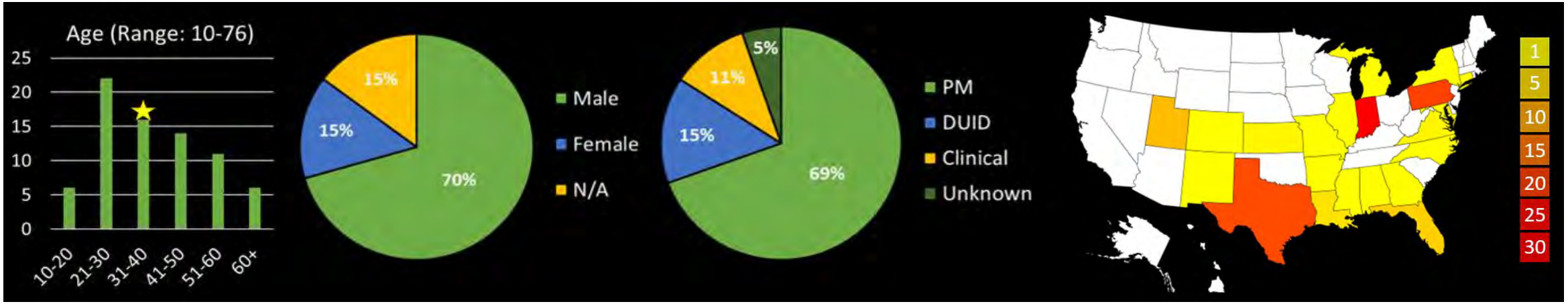
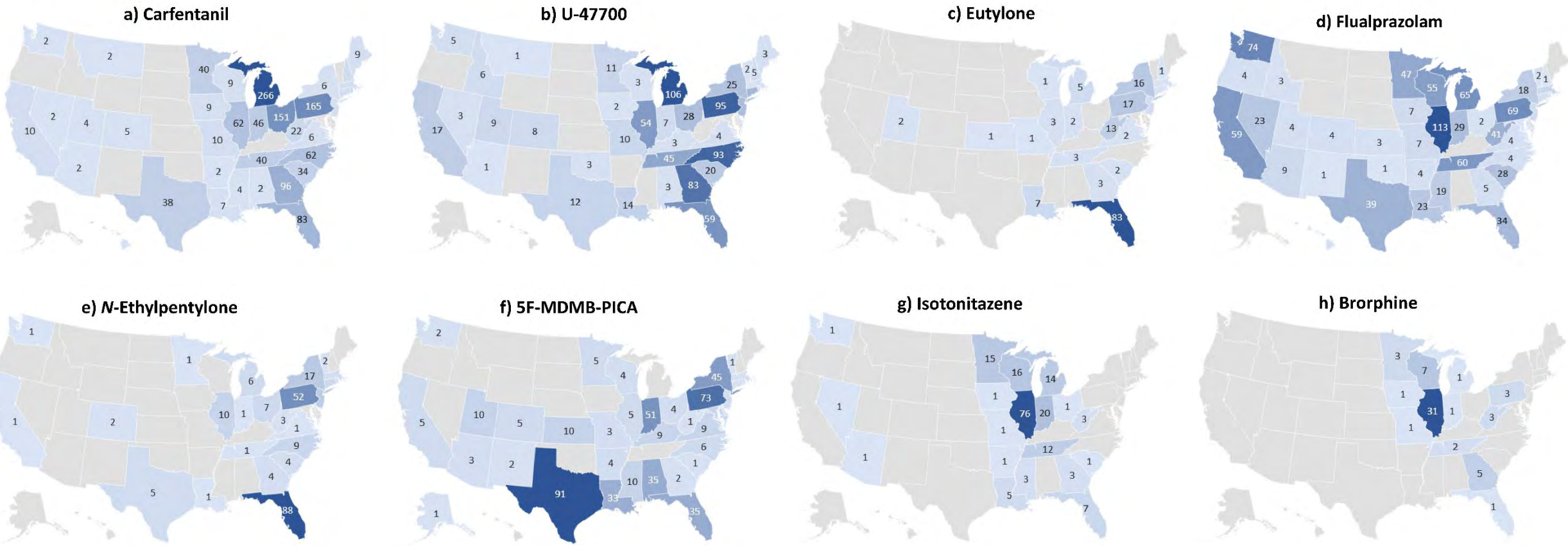
New Drugs Entering the United States

- Example 1: 2-Methyl AP-237 (opioid)
- Example 2: 4F-MDMB-BINACA (cannabinoid)



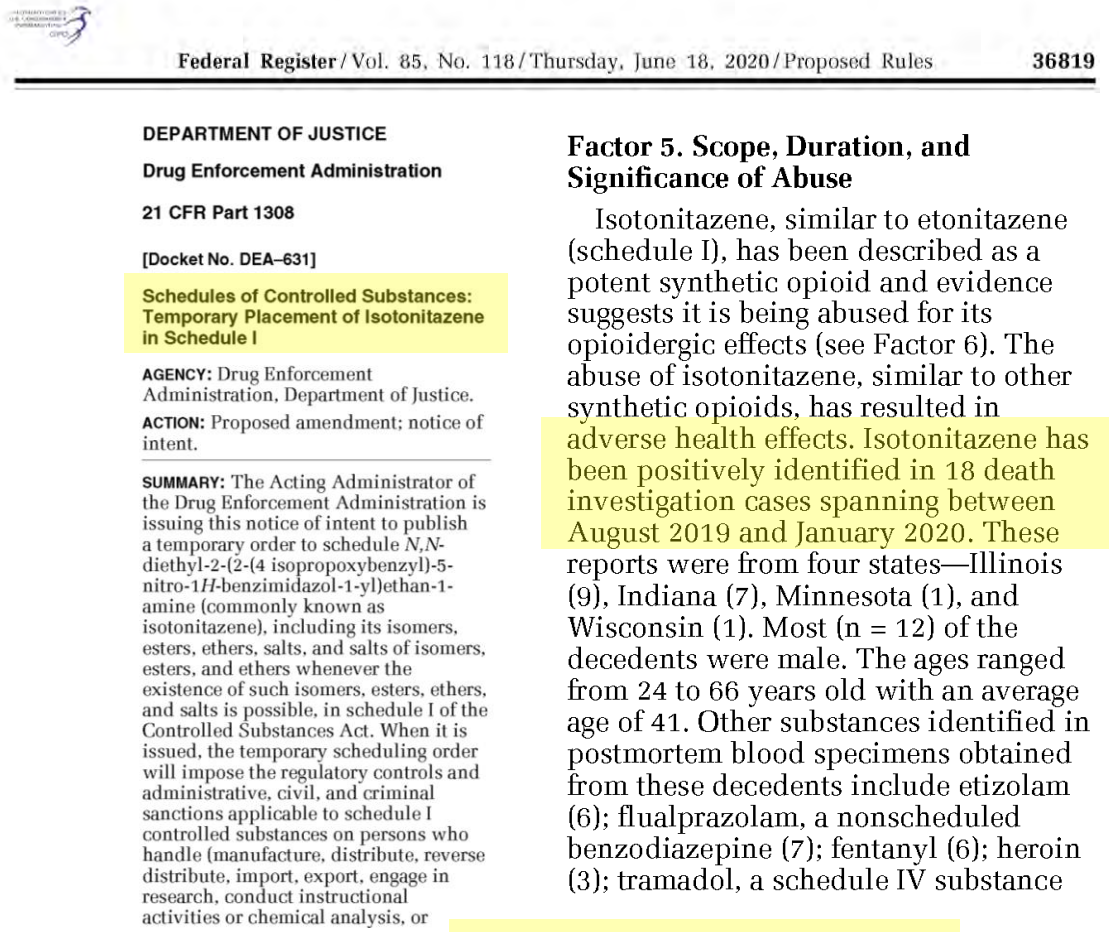


Geographical Differences Observed



DEA Scheduling of New Synthetic Drugs

- **8-Factor Analysis:**
  1. Its actual or relative potential for abuse.
  2. Scientific evidence of its pharmacological effect, if known.
  3. The state of current scientific knowledge regarding the drug or other substance.
  4. **Its history and current pattern of abuse.**
  5. **The scope, duration, and significance of abuse.**
  6. **What, if any, risk there is to the public health.**
  7. Its psychic or physiological dependence liability.
  8. Whether the substance is an immediate precursor of a substance already controlled under this subchapter.



Federal Register / Vol. 85, No. 118 / Thursday, June 18, 2020 / Proposed Rules 36819

DEPARTMENT OF JUSTICE

Drug Enforcement Administration

21 CFR Part 1308

[Docket No. DEA–631]

Schedules of Controlled Substances: Temporary Placement of Isotonitazene in Schedule I

AGENCY: Drug Enforcement Administration, Department of Justice.

ACTION: Proposed amendment; notice of intent.

SUMMARY: The Acting Administrator of the Drug Enforcement Administration is issuing this notice of intent to publish a temporary order to schedule *N,N*-diethyl-2-(2-(4 isopropoxybenzyl)-5-nitro-1*H*-benzimidazol-1-yl)ethan-1-amine (commonly known as isotonitazene), including its isomers, esters, ethers, salts, and salts of isomers, esters, and ethers whenever the existence of such isomers, esters, ethers, and salts is possible, in schedule I of the Controlled Substances Act. When it is issued, the temporary scheduling order will impose the regulatory controls and administrative, civil, and criminal sanctions applicable to schedule I controlled substances on persons who handle (manufacture, distribute, reverse distribute, import, export, engage in research, conduct instructional activities or chemical analysis, or

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

[8] Krotulski AJ, Papsun DM, Kacinko SL, and Logan BK (2020). Isotonitazene Quantitation and Metabolite Discovery in Authentic Forensic Casework. *Journal of Analytical Toxicology*. [Epub ahead of print].

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Poly-Drug Use

- Poly-drug use continues to increase, especially with opioids and benzodiazepines
  - Toxicology testing vs. drug material testing
- It is rare to find single-drug toxicology cases involving NPS
  - Drug products are more frequently mixed, cut, diluted, adulterated, etc.
  - Toxicology testing has become more comprehensive
  - Complicates interpretation
- Common combinations:
  - Benzodiazepines and fentanyl
  - Fentanyl and stimulants (coc. and/or meth.)
  - NPS opioids and NPS benzodiazepines

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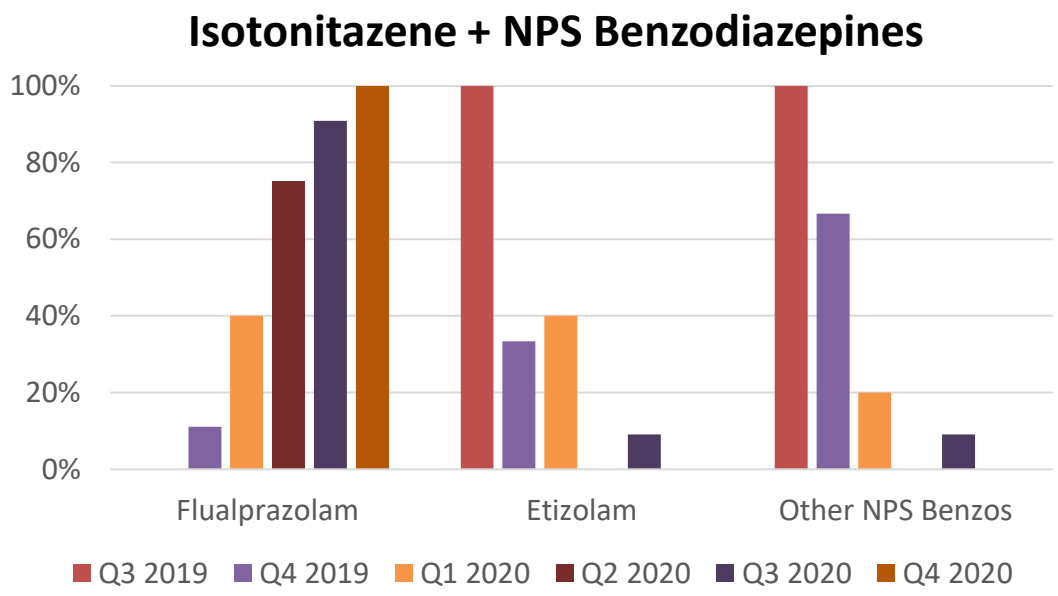
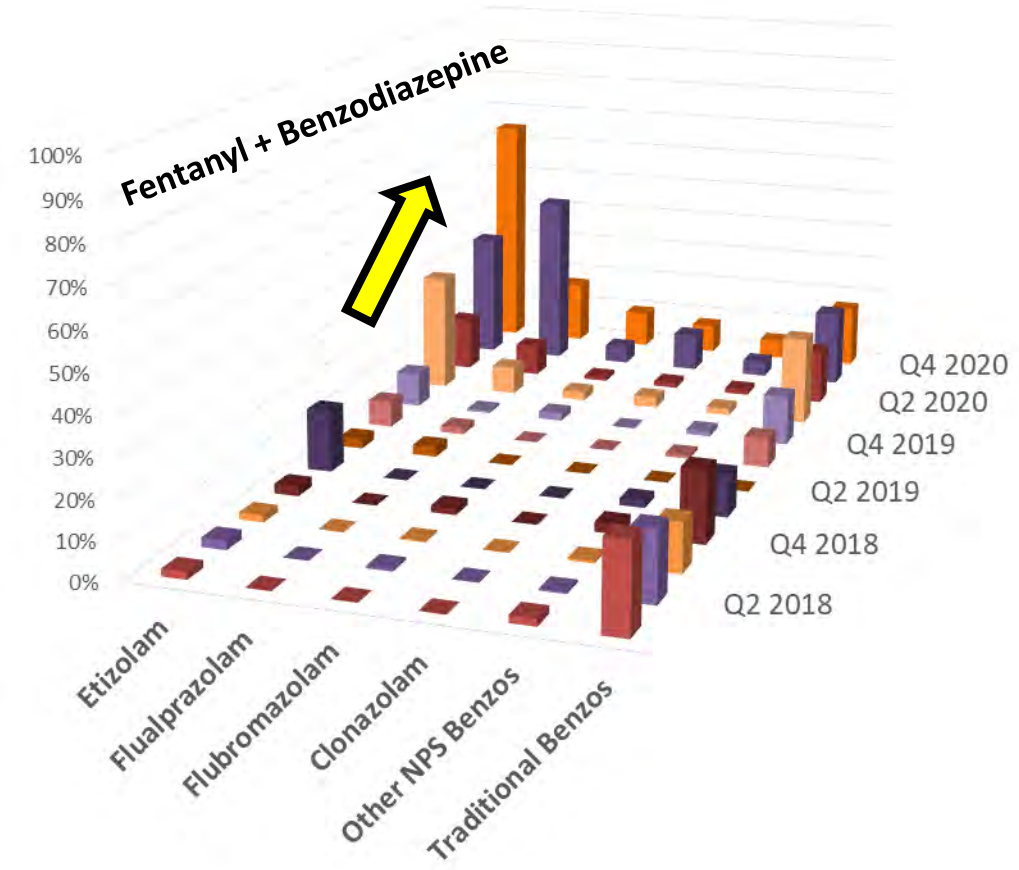
Concurrent Use: Benzodiazepines and Opioids

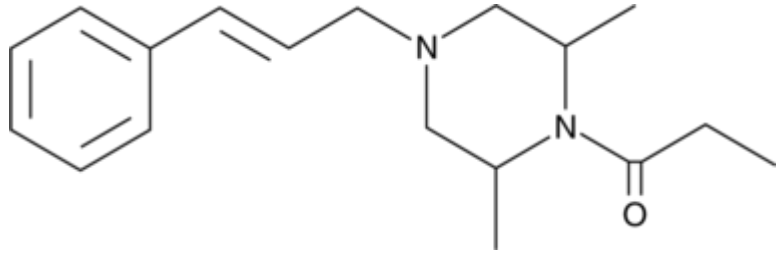
- NPS benzodiazepines are increasingly being found in combination with opioids (mostly fentanyl)





Crude Pharmacological Assessments?

- **Real Case Example:**
  1. Medical examiner reaches out about a death
  2. Routine toxicology testing is negative (no cause/manner of death)
  3. Autopsy shows signs of suspected drug death (e.g., pulmonary edema)
  4. Toxicology samples sent for expanded testing
  5. New opioids “AP-238” discovered
  6. Laboratory quantifies the drug in blood sample



- **Interpretation:** Active drug, Opioid-like, Less potent than fentanyl*

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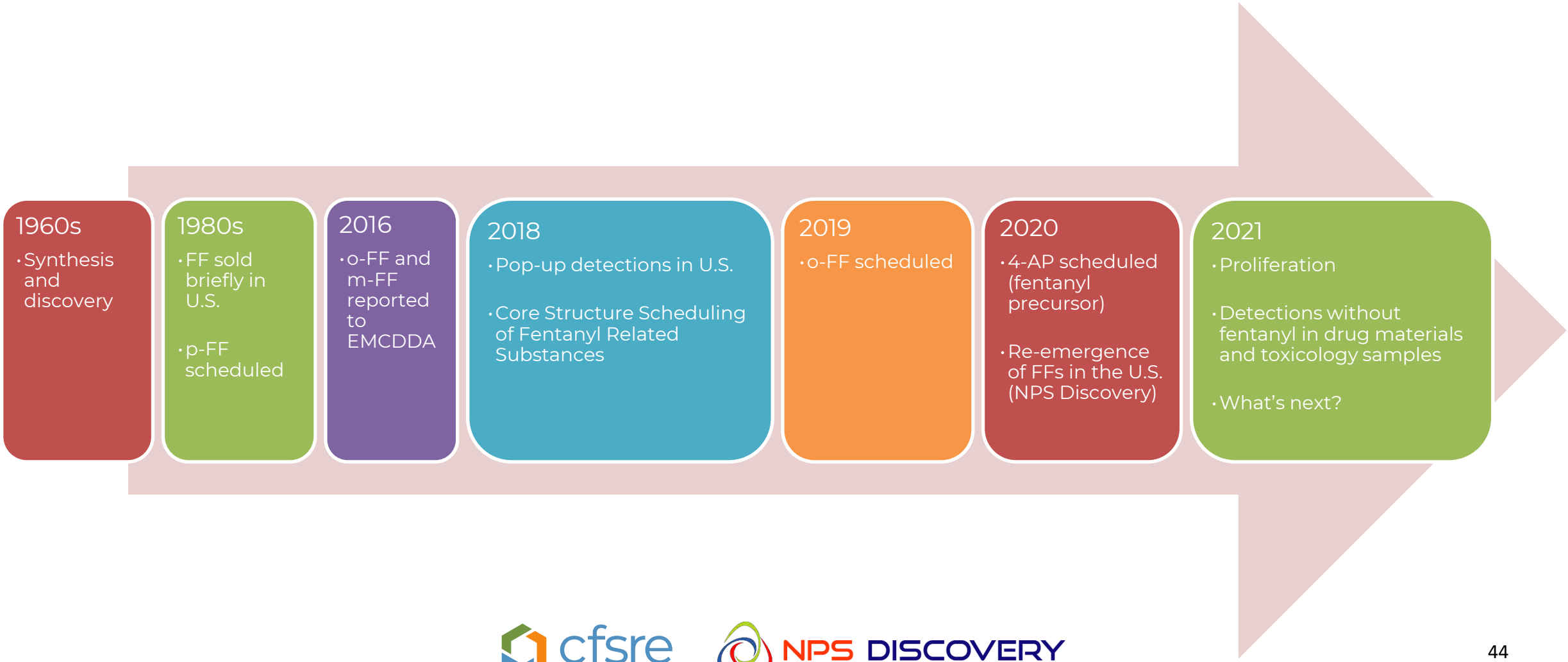
Timeline of Fluorofentanyl



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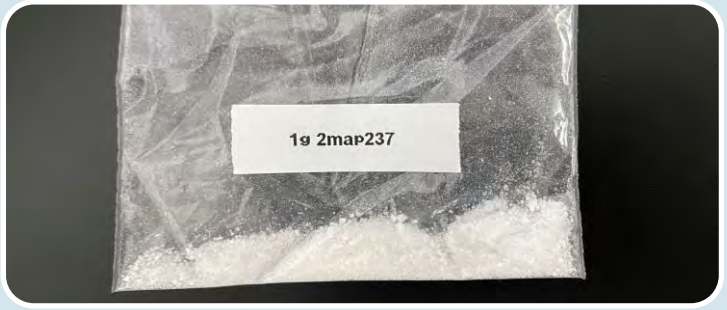
But … Not All NPS Opioids Are Created Equal



Gray Market Vendors & Research Chemicals

I.e., buying drugs online

E.g., 2-Methyl AP-237

Sold Recreational as "Heroin/Fentanyl"

I.e., buying drugs on street

E.g., Metonitazene (Midwest)

Combined With The Larger Drug Supply*

I.e., buying drugs on street

E.g., para-Fluorofentanyl with Fentanyl (Widespread)

**Excluding cases where drug found alone*

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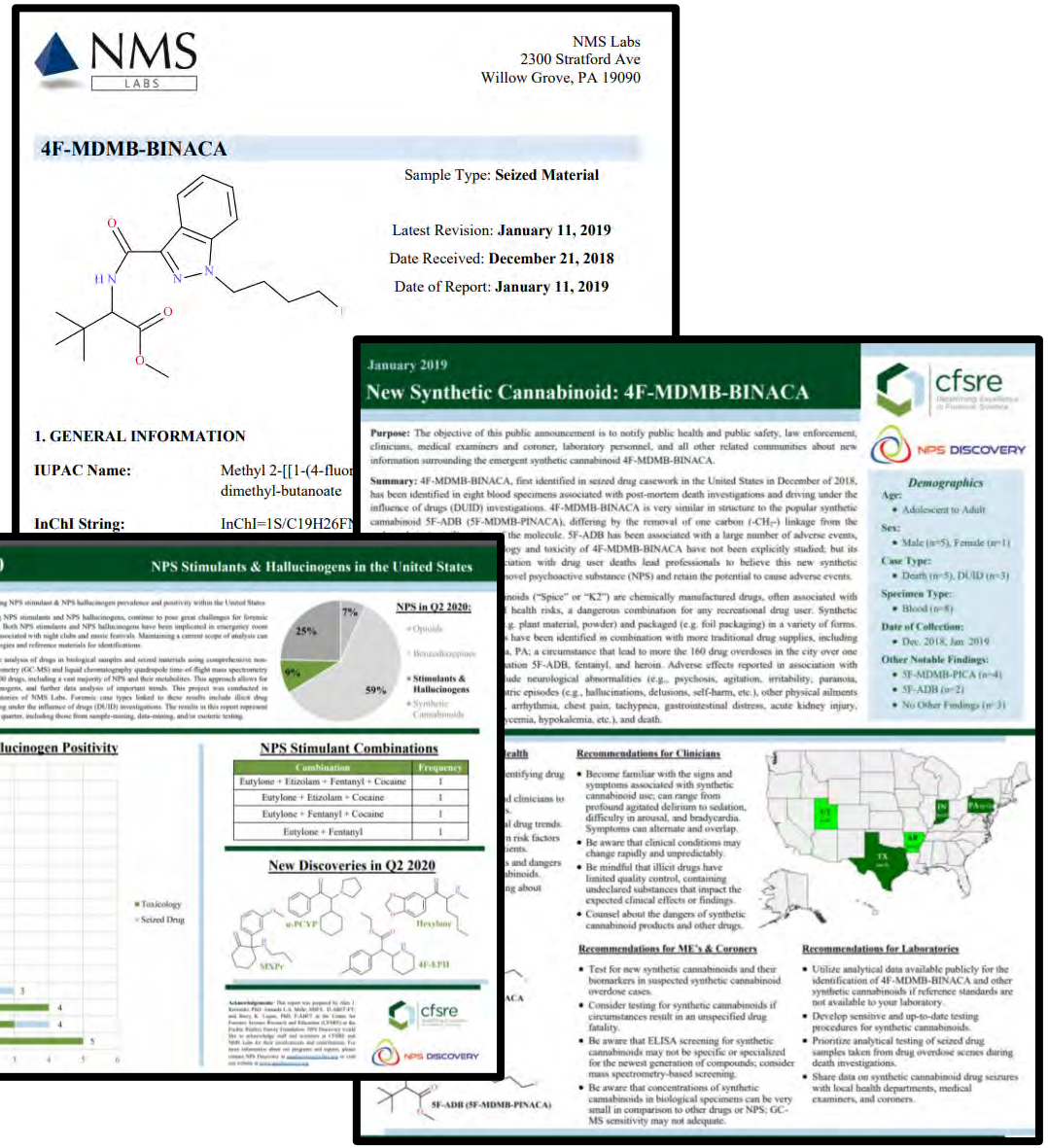
New Drug Monographs

Public Alerts

Scientific Publications

Trend Reports

And So Much More !!!



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

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TAKE AWAYS

Persistent Challenges

- Synthetic drugs present in forensically relevant samples often remain **unidentified** due to scope of analysis and analyst experience/expertise

- First identification are often **lagging or delayed**
  - Analytical capabilities, testing performed
  - Standard reference material
  - Relation to previously identified NPS

- There is **no centralized reporting system** within the United States

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Considerations for Identifying NPS

- **Sample Source:**
  - Understand population, effects, etc.
  - Are you testing the correct matrix?
- **Analytical Methods:**
  - Mass spectrometry
  - Sensitive and specific instruments
  - Non-targeted data acquisition

- **Discovery Workflows:**
  - Does your laboratory have a strategy?
    - NPS do not just *appear*
  - Non-targeted data processing
  - Appropriate acceptance criteria
- **Results & Outcomes:**
  - Public and private partnerships foster generation of important data
  - Dissemination is key!
  - Forecasting future trends?

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Recommendations

- Implement data independent acquisition (“non-targeted”) strategies
- Use high resolution mass spectrometry for drug screening
- Develop appropriate data processing workflows
- Maintain updated and accurate scopes of testing
- Partner with laboratories outside your field for testing
- Collaborate with other scientists to develop meaningful results
- Dissemination and information sharing!
- Seek opportunities for funding and support

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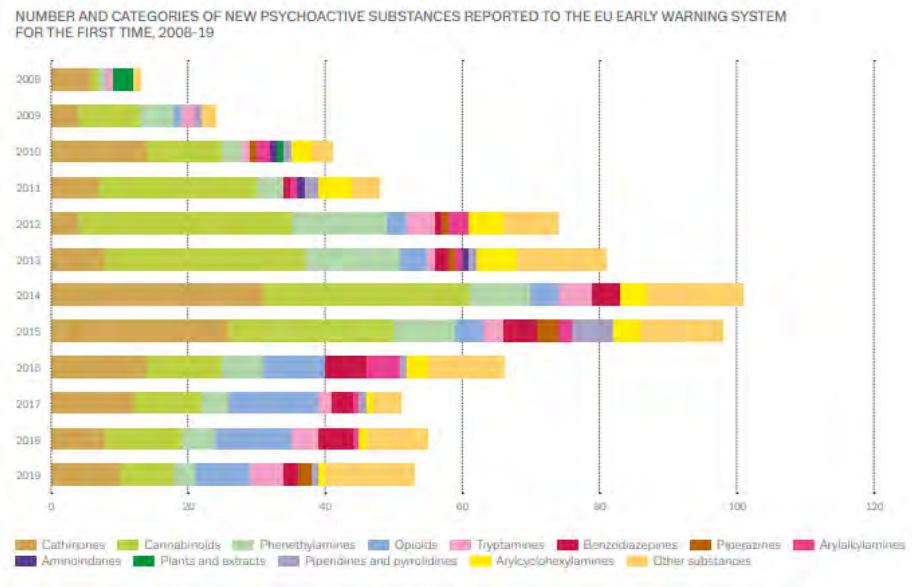
Conclusions

- Drug testing for new synthetic substances is not easy… and it takes an army
- Constantly evolving NPS trends lead to constant need for adjusting/adapting
- Expertise and advanced skill sets are needed
- State-of-the-art instrumentation is preferred
- Resources are available
  - Method development, test design, etc.



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  - Carolina Noble
- **Forensic and Clinical Partners**
- **Medical Examiner and Coroner Partners**
- **Federal, State, and Local Partners**

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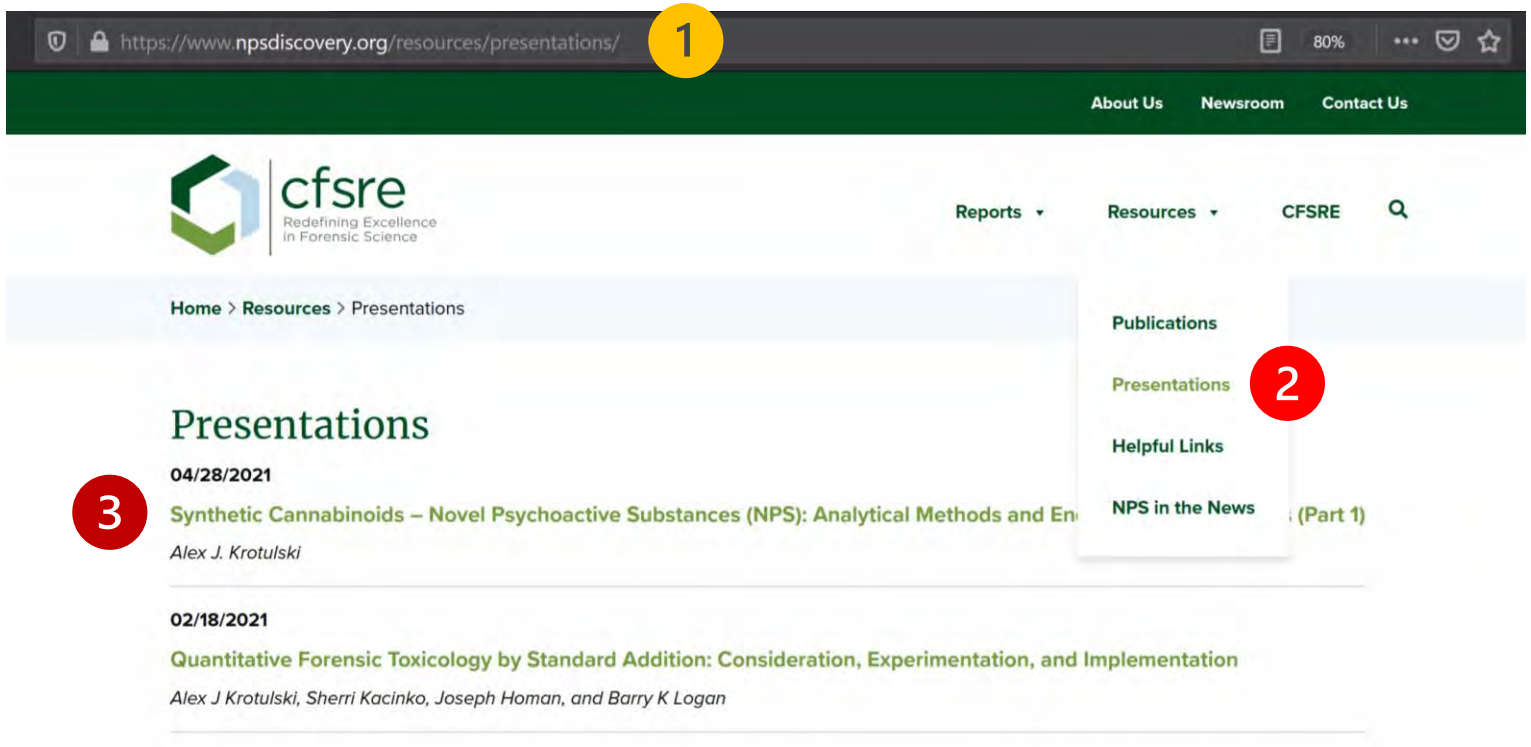

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