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# Implementation of NPS Discovery – An Early Warning System for Novel Drug Intelligence, Surveillance, Monitoring, Response, and Forecasting using Drug Materials and Toxicology Populations in the US

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## PROJECT SUMMARY

### 1. Goals and Objectives

This research addressed one of the greatest technical challenges facing toxicologists and chemists in the forensic field today: keeping up to date with the most current drugs and novel psychoactive substances (NPS). During this award, our laboratory built on previous research funded by the National Institute of Justice (NIJ) on method development, validation, and application to apply novel tools to the identification of emerging drugs, to characterize these drugs in forensic toxicological and chemistry samples, and to share methods, analytical data, toxicological data, and more with the forensic practitioner community. This project helped to facilitate and ensure more accurate and timely reporting of new drugs, aid scheduling and control actions, and assist with interdiction efforts and drug trafficking intelligence. The continuation of this project from prior funding allowed our laboratory to continue applying novel tools and techniques to develop enhanced workflows for rapid identification of new and emerging drugs and to rapidly disseminate information to various stakeholders.

*1.1 Purpose, Aims, and Objective*

The purpose of this research was to use sample-mining and data-mining efforts to detect changes in NPS markets and develop workflows for use by forensic science practitioners as limited information is available on emerging NPS, particularly involving reference concentration data, analytical data, trends, and pharmacology. This research was divided into six key objectives:

1. **NPS Surveillance:** Develop and upkeep a continuously up-to-date testing panel using an expansive library database to include new drugs available from standard reference material manufacturers to create the most accurate and comprehensive scope available.
2. **NPS Monitoring:** Using sample-mining and data-mining approaches, conduct extensive testing of authentic forensic samples to detect and discover the newest drugs present within forensically relevant populations, as well as to determine the most prevalent drugs requiring further research and developments.
3. **NPS Metabolism:** Characterize metabolic pathways associated with new and prevalent NPS to identify the most appropriate biomarkers to assist with future testing, and to determine if generated metabolites could contribute to effects or toxicity.
4. **NPS Confirmation:** Rapid development and validation of confirmatory testing methods for these NPS in toxicology samples using various vendor platforms to establish reference concentration data with a specific focus on developing methods that can be directly transferrable to forensic laboratories.
5. **NPS Toolkits:** Consolidate research outcomes to generate a comprehensive document (or "toolkit") detailing relevant information regarding the detection of NPS, including basic drug information, demographics, date of first appearance, prevalence, temporal and geographic trends, combinations with other NPS or drugs of abuse, metabolism, methods for confirmations, and/or reference concentrations.

6. **Knowledge Transfer:** Disseminate deliverables from this research to forensic science and criminal justice stakeholders via email and through a website. Presentations at in-person and online forensic science conferences are part of our dissemination strategy, as well as peer reviewed publication of key findings in appropriate forensic science journals with a focus on open-access options.

The project described allowed for rapid identification and dissemination of information to combat emerging drug threats, including NPS. This approach provides resources that were previously unavailable to other forensic laboratories performing biological sample (driving under the influence of drugs (DUID) or medicolegal death investigation cases) or drug material testing. This endeavor relieves the burden on practicing laboratories as it gave framework for method development and validation of these new drugs and allowed ease of identification with the provided data.

## 2. Research Questions

### *2.1 Research & Development Needs*

Recreational and illicit use of drugs in the United States have been major contributors to morbidity and mortality among vulnerable and high-risk populations (1). Many United States agencies such as the Organization of Scientific Area Committees (OSAC) at the National Institute of Standards and Technology (NIST) and the NIJ have expressed the need for greater research and development in toxicology and chemistry (2). Specifically, these agencies have described the need for research involving emerging drugs causing overdoses and fatalities (i.e., NPS), particularly involving developing analytical workflows for the characterization of NPS (e.g., quantitative methods, metabolism studies, and postmortem redistribution studies). These needs have been addressed with this research to allow for the continued development of knowledge regarding NPS.

This project included objectives that provided workflows and tools to produce accurate, immediate, and easily usable information to ease identification of NPS. In turn, this allowed for more informed drug policy for the scheduling of NPS in federal, state, and local laws. The deliverables produced and distributed as part of this project have direct application in practicing forensic laboratories, including (but not limited to) the generation of drug monographs, trend reports, public alerts, peer reviewed analytical methods, workflows, and ultimately culminating as toolkits to promote adoption in practicing forensic laboratories.

### *2.2 Knowledge Gaps*

There remain significant knowledge gaps in relation to NPS because it is often unknown what the true extent of NPS involvement in forensic investigations is due to misidentification or under-reporting of NPS. Other research and forensic testing workflows do not allow for simple addition of NPS to testing scopes and therefore do not allow for accurate characterization of NPS effects on public health and safety. Many forensic toxicology and chemistry laboratories do not have the capabilities to keep up to date with the constantly evolving drug landscape; however,

there is a need to rapidly characterize NPS (i.e., chemical signature, potency, activity, toxicity, metabolism). NPS causing fatal and non-fatal overdoses show significant risk to public health and quick development of accurate toxicology testing panels is imperative to ensure that appropriate drugs and biomarker(s) can be identified and incorporated into drug testing methods and potentially provide information for drug legislation.

*2.3 Importance of Research & Criminal Justice Significance*

This research filled the knowledge gap described above as it provided knowledge critical to forensic science practitioners to ensure early detection of NPS. The research provided workflows and tools to identify new drugs contributing to illicit drug trafficking, overdoses, deaths, and injuries in the United States, as there is currently no comprehensive national early warning system for new drug identification (3). Outside of the United States, there are programs that provide drug-related information based on drug trends and drug use, specifically, the European Union Drugs Agency (EUDA), which operates a drug early warning system (4). The EUDA has successfully identified the emergence of more than 500 NPS using this system (5).

Many organizations need timely information on emerging drugs; however, prior to this research, the information was generally not timely or comprehensive. Forensic and criminal justice stakeholders greatly benefit from the rapid discovery and dissemination of information to drug chemists, toxicologists, medical examiners, coroners, and law enforcement (6). As NPS have been associated with deaths and adverse events, the identification and reporting of NPS has been shown to be a key contributor in public health, public safety, and the support of drug legislation through the criminal justice system.

*2.4 Impacts*

This research project significantly improved scientific knowledge and technical capabilities for NPS through method development, validation, characterization, and implementation on many instrumental platforms. The rapid dissemination of information for newly identified NPS raised awareness of the threats that NPS pose to the United States. The prompt development of methods and characterization through metabolism studies expanded technical capabilities for forensic laboratories and provided opportunities to make existing analytical approaches targeted and relevant to prevalent NPS.

Specific deliverables from this project were drug characteristics and structural information to assist with drug scheduling actions, quantitative methods for NPS, metabolite determination for appropriate biomarker testing through *in vitro* and *in vivo* studies, and reference concentration data from fatalities related to NPS. Dissemination of analytical data and additional drug information (e.g., trend reports to allow for appropriate scope of testing) were provided through an open-access electronic database. Reported drug concentration data, drug co-morbidities, and outcome data (e.g., cause and manner of death) assisted toxicologists, pathologists, and medical examiners, as well as physicians, medical toxicologists, and drug enforcement agents, in their work supporting public health and safety.

## 3. Summary of Project Design and Methods

### *3.1 Objective 1: NPS Surveillance*

The intent of this objective was to maintain an up-to-date and expansive library database and testing panel to include new drugs and NPS that became available through standard reference material manufacturers. Two liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) systems were used for discovery during this research as previous NIJ funded research (2015-IJ-CX-K012, 2017-R2-CX-0021, 2020-DQ-BX-0007) (7, 8) and forensic laboratory implementation have demonstrated LC-QTOF-MS (or more broadly, high resolution mass spectrometry) to be the most accurate and powerful analytical tool for comprehensive drug and NPS identification in drug materials and biological samples, specifically in comparison to other technologies. LC-QTOF-MS provided the sensitivity, specificity, and certainty required for NPS analysis through the acquisition of complex multi-stage mass spectral data.

To maintain the expansive library database, the primary activity under this objective was to analyze newly available standard reference materials as comparison to a known standard is required for the reporting of reliable results. Standard reference materials were purchased from qualified reference material vendors. Appropriate additions to the database were determined based on intelligence from collaborators and colleagues, as well as government drug scheduling actions, illicit drug market activity, and more.

Drug standards were analyzed by LC-QTOF-MS prior to addition to the library database, which allowed for verification of drug standards and monitoring of NPS in forensic casework. Analysis was conducted using either a Sciex TripleTOF™ 5600+ or a X500R LC-QTOF-MS. Methods on both instruments were replicated to allow for use of either instrument for drug standard and/or authentic case analysis. Reverse phase gradient elution was utilized with mobile phases of ammonium formate (10 mM, pH 3) [MPA] and acetonitrile/methanol (0.1% formic acid, 50:50 *v:v*) [MPB] and an instrumental gradient beginning at 95:5 A:B and increasing to 5:95 A:B over 13 minutes with a total run time of 15.5 minutes (9). A Phenomenex Kinetex C18 column (50 x 3 mm, 2.6 µm) at 30°C was used and the QTOF was operated in positive electrospray ionization mode (ESI+).

The gradient described above was used for library additions and authentic sample analysis; however, the QTOF was operated under information dependent acquisition (IDA) for library addition and variable window SWATH acquisition for authentic samples. IDA allowed for generation of fragment spectra for the drug standards with highest specificity and fragmentation was achieved using a rolling collision energy of 35±15eV. The fragment spectrum produced with IDA allowed for universal use with IDA or SWATH methodology.

The library database was maintained in-house, and this included the updating of a mass list (or extracted ion chromatogram [XIC] list) and library file of fragment spectra. The XIC list includes several pieces of data from the acquired standards, such as retention time, formula, exact mass, accurate fragment masses (n= up to 5 fragments), and general software required settings. This information will be compiled and consolidated in an Excel spreadsheet and into the

instrument library software (LibraryView). Data processing was performed using Sciex OS (Version 3.3), PeakView (Version 2.2), MasterView™ (Version 1.1), and LibraryView (Version 1.0) – four software applications available from Sciex, the instrument manufacturer.

*3.2 Objective 2: NPS Monitoring*

To determine the positivity and prevalence of NPS, this research included testing authentic specimens for the identification of new drugs to determine NPS impact on the United States. To monitor NPS, our laboratory performed sample-mining (i.e., non-targeted retesting of samples or sample extracts) and partnered with other laboratories to acquire biological specimens and sample extracts from forensic (DUID, medicolegal death investigations), clinical (non-fatal overdoses, outbreaks), and seized drug casework. These samples were prepared using two single-step liquid-liquid extraction (LLE) methods. Basic LLE was performed using borax buffer (0.1 M, pH 10.4, 1 mL) and N-butyl chloride/ethyl acetate (70:30, v:v, 3 mL) and acidic LLE was performed using phosphoric acid (1 mL, 5% in water) and an extraction solvent of hexane/ethyl acetate/methyl tert-butyl ether (3 mL, 80:10:10, *v:v:v*) (10). Sample extracts were analyzed "as received."

LC-QTOF-MS methods were developed and validated for identification and characterization of NPS from biological samples as described above (variable window SWATH acquisition, non-targeted analysis) and under previous NIJ funding (2015-IJ-CX-K012 and 2017-R2-CX-0021) (7, 8). Methods were validated using AAFS Standards Board (ASB) Standard Practices for Method Validation in Forensic Toxicology (ASB 036) (11). The LC-QTOF-MS methodology developed demonstrated necessary accuracy and robustness for the identification of NPS and is a general, comprehensive screen for the detection of toxicologically significant substances, including drugs of abuse, therapeutic drugs, cutting agents, incidental substances, NPS, and metabolites. NPS classes incorporated into this method included opioids, stimulants, hallucinogens, benzodiazepines, and cannabinoids; however, due to their unique chemistry, the method is not well optimized for the detection of synthetic and semi-synthetic cannabinoids. For this reason, a secondary screen was designed to target synthetic cannabinoids and their metabolites. Due to rapidly changing NPS markets, both methods require constant re-optimization and expansion; however, re-development and re-validation are not required.

Data processing was performed as described above with vendor software, including Sciex OS (Version 3.3) and MasterView™ (Version 1.1), which allow for large batch processing of samples (i.e., up to 200 samples) and in-batch comparison of sample-to-control or sample-to-sample features. Data processing was conducted against the most current in-house library database as developed according to the process above.

*3.3 Objective 3: NPS Metabolism*

The intent of this objective was to use *in vitro* and *in vivo* studies to determine or predict the metabolism of emerging NPS. Accurate characterization was imperative as this information can be used to update forensic scopes of testing with appropriate biomarkers for NPS of interest. Human liver microsomal incubation was used to assess in vitro metabolism of NPS, which produced ~20 metabolic species per drug; however, further confirmation of the metabolites in

authentic biological specimens was completed if available. Confirmation of metabolites in authentic blood and urine was performed by analysis of cases where the NPS was confirmed to be ingested and allowed for correlation between in vitro and in vivo testing. Analysis of a population of biological samples was tested if available to determine prevalence of NPS metabolites in humans, which assists forensic laboratories in keeping up-to-date scopes of testing without including unnecessary drugs or metabolites.

HLM incubations were performed for NPS of interest (substances that were identified as prevalent during previous objectives) alongside a well-characterized traditional drug (diazepam). For the metabolism studies, standards, controls, and metabolism reaction mixtures were prepared with varying volumes of phosphate buffer (10 mM, pH 6), drug of interest (1 mg/mL), NADPH (co-factor), and HLM in accordance with previously published studies (12). To ensure accuracy of results and to minimize intra-day variability, experiments were conducted in replicates on multiple days for an appropriate sample size. Samples were analyzed via LC-QTOF-MS using both targeted and non-targeted acquisition modes (as described above in Objectives 1 and 2). Authentic human biological specimens were obtained from collaborators for confirmation of metabolites generated in the above experiments.

Data processing was performed using a variety of software applications available from the instrument vendor (e.g., MetabolitePilot™, PeakView®, MasterView™). A multistage processing workflow will be employed, including aspects of identification, verification, and confirmation. In brief, MetabolitePilot™ was used to process the acquired data from HLM experiments and MasterView™ was used for batch processing and review of authentic specimens. Information for the metabolites identified was compiled using Excel to tabulate pieces of data such as accurate mass, accurate fragment masses, retention time, and structure.

*3.4 Objective 4: NPS Confirmation*

In addition to qualitative surveillance of NPS using LC-QTOF-MS, our laboratory developed quantitative methodologies for prevalent NPS using instrumentation commonly encountered in forensic laboratories (e.g., liquid chromatography tandem quadrupole mass spectrometry [LC-QQQ-MS]). Our laboratory partnered with other forensic laboratories, medical examiner/coroner's offices, police departments, and other entities to obtain authentic human biological specimens from forensic investigations.

Three confirmation methods for NPS were developed, validated, and published during the project period. LLE and solid phase extraction (SPE) protocols were developed and evaluated to determine the optimal extraction procedure for each NPS panel and methods were validated according to ASB Standard 036 (11). The validation for quantitative analysis included quantitative confirmation (i.e., calibration curves, control samples), and evaluation of accuracy, precision, carryover, limit of detection, limit of quantitation, dilution integrity, and interferences from matrix, drug, internal standard, and commonly encountered analytes. Standard addition was evaluated in place of external calibration if necessary. Analyte recovery was evaluated using a pre-spike and post-spike experiment. These methods were generally developed for blood, which is the gold standard for quantitative analysis in forensic toxicology; however, urine, serum,

plasma, oral fluid, and other alternative matrices were included in the validation and accepted for analysis if available.

Methods for the confirmation and quantitation of *N,N*-dimethylpentylone (and related synthetic cathinones), dipyanone, and new nitazene analogues were developed and published during the project period (10, 13, 14). Additional panels for *ortho*-methylfentanyl, medetomidine, xylazine, and other NPS are pending publication. NPS panels were developed based on emerging NPS discovered during surveillance and monitoring efforts and were adapted as necessary. As described above, sample preparation was performed via optimal basic or acidic LLE and instrumental analysis was completed using LC-QQQ-MS in ESI+ using multiple reaction monitoring (MRM). Optimal LC and MS parameters were determined for each drug prior to validation. Authentic samples were acquired from submitting agencies (e.g., forensic laboratories, medical examiner/coroner's offices, police departments, etc.). Data were consolidated into Excel spreadsheets prior to publication in peer-reviewed journals.

## 4. Summary of Results

### *4.1 Objective 1: NPS Surveillance*

One hundred and thirteen drug standards were added to the scope of analysis during the research period of 2023 to 2024 (**Table 1**). This database expansion exceeded the original goal of 50-100 new NPS based on the historical emergence of new NPS. Using the expanded library database for the surveillance of new NPS, 37 new drug monographs (**Table 2**) and four NPS scope recommendations were issued (https://www.cfsre.org/nps-discovery/scope-recommendations). The addition of drug standards allowed for surveillance and monitoring of new NPS that were previously unknown or uncharacterized by forensic scientists.

**Table 1:** New drug standards added to library database.

| # | Drug Name | Chemical Formula | [M+H]+ (Da) | Source |
|---|---|---|---|---|
| 1 | MDMB-5'Br-BUTINACA | $C_{19}H_{26}BrN_3O_3$ | 424.1230 | Cayman Chemical |
| 2 | *N*-Pyrrolidino 4'-hydroxy Nitazene | $C_{20}H_{22}N_4O_3$ | 367.1765 | Cayman Chemical |
| 3 | *N*-Piperidinyl 4'-hydroxy Nitazene | $C_{21}H_{24}N_4O_3$ | 381.1921 | Cayman Chemical |
| 4 | (±)-9β-hydroxy HHC | $C_{21}H_{32}O_3$ | 333.2424 | Cayman Chemical |
| 5 | (±)-9α-hydroxy HHC | $C_{21}H_{32}O_3$ | 333.2424 | Cayman Chemical |
| 6 | 9(R)-HHC Acetate | $C_{23}H_{34}O_3$ | 359.2581 | Cayman Chemical |
| 7 | 9(R)-HHC | $C_{21}H_{32}O_2$ | 317.2475 | Cayman Chemical |
| 8 | 9(R)-HHCP | $C_{23}H_{36}O_2$ | 345.2788 | Cayman Chemical |
| 9 | 9(S)-HHC | $C_{21}H_{32}O_2$ | 317.2475 | Cayman Chemical |
| 10 | Dexmedetomidine | $C_{13}H_{16}N_2$ | 201.1386 | Cayman Chemical |
| 11 | 11-COOH-HHC | $C_{21}H_{30}O_4$ | 347.2217 | Cayman Chemical |
| 12 | Cannabinol (CBN) | $C_{21}H_{26}O_2$ | 311.2006 | Cayman Chemical |
| 13 | Cannabigerol (CBG) | $C_{21}H_{32}O_2$ | 317.2475 | Cayman Chemical |
| 14 | Cannabidiol (CBD) | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 15 | Δ9-THC Acetate | $C_{23}H_{32}O_3$ | 357.2424 | Cayman Chemical |
| 16 | (6aR,9R)-Δ10-THC | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 17 | 9(S)-Δ6a,10a-THC | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 18 | (−)-11-hydroxy-Δ8-THC | $C_{21}H_{30}O_3$ | 331.2268 | Cayman Chemical |

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| 19 | (±)-11-nor-9-carboxy-Δ9-THC | $C_{21}H_{28}O_4$ | 345.2060 | Cayman Chemical |
| 20 | (±)-11-hydroxy-Δ9-THC | $C_{21}H_{30}O_3$ | 331.2268 | Cayman Chemical |
| 21 | Δ9-THCP | $C_{23}H_{34}O_2$ | 343.2632 | Cayman Chemical |
| 22 | 9(S)-Δ7-THC | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 23 | exo-THC | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 24 | Lormetazepam | $C_{16}H_{12}Cl_2N_2O_2$ | 335.0349 | Cerilliant |
| 25 | ((±)-4-MeTMP) | $C_{15}H_{21}NO_2$ | 248.1645 | Cayman Chemical |
| 26 | *N*-Pyrrolidino Isotonitazene | $C_{23}H_{28}N_4O_3$ | 409.2234 | Cayman Chemical |
| 27 | *N*-methyl-*N*-propyl Methylone | $C_{14}H_{19}NO_3$ | 250.1438 | Cayman Chemical |
| 28 | *N*-butyl Butylone | $C_{15}H_{21}NO_3$ | 264.1594 | Cayman Chemical |
| 29 | Despropionyl *para*-Fluorofentanyl | $C_{19}H_{23}FN_2$ | 299.1918 | Cayman Chemical |
| 30 | Ketamine hydroxylimine precursor | $C_{13}H_{16}ClNO$ | 238.0993 | Cayman Chemical |
| 31 | *N*-desethyl Protonitazene | $C_{21}H_{26}N_4O_3$ | 383.2078 | Cayman Chemical |
| 32 | Δ8-iso-THC | $C_{21}H_{30}O_2$ | 315.2319 | Cayman Chemical |
| 33 | 25B-NBOH | $C_{17}H_{20}BrNO_3$ | 366.0699 | Cayman Chemical |
| 34 | Ethyleneoxynitazene | $C_{22}H_{26}N_4O_3$ | 395.2078 | Cayman Chemical |
| 35 | MDMB-BUTINACA | $C_{19}H_{27}N_3O_3$ | 346.2125 | Cayman Chemical |
| 36 | AMB | $C_{19}H_{27}N_3O_3$ | 346.2125 | Cayman Chemical |
| 37 | Phenylbutazone | $C_{19}H_{20}N_2O_2$ | 309.1598 | Cayman Chemical |
| 38 | Aminophenazone | $C_{13}H_{17}N_3O$ | 232.1444 | Cayman Chemical |
| 39 | Rilmazafone | $C_{21}H_{20}Cl_2N_6O_3$ | 475.1047 | Toronto Research Chemicals |
| 40 | Metamizole (Dipyrone) | $C_{13}H_{16}N_3O_4S$ | 311.0934 | Cayman Chemical |
| 41 | *alpha*-hydroxy Bromazolam | $C_{17}H_{13}BrN_4O$ | 369.0345 | Cayman Chemical |
| 42 | N-Pyrrolidino Metodesnitazene | $C_{21}H_{25}N_3O$ | 336.2070 | Cayman Chemical |
| 43 | Desalkylquazepam | $C_{15}H_{10}ClFN_2S$ | 305.0310 | Cayman Chemical |
| 44 | 4-fluoro AB-BUTINACA | $C_{17}H_{23}FN_4O_2$ | 335.1878 | Cayman Chemical |
| 45 | *N*-propyl Buphedrone | $C_{13}H_{19}NO$ | 206.1539 | Cayman Chemical |
| 46 | Nitemazepam | $C_{16}H_{13}N_3O_4$ | 312.0979 | Cayman Chemical |
| 47 | *N*-desethyl Metonitazene | $C_{19}H_{22}N_4O_3$ | 355.1765 | Cayman Chemical |
| 48 | Ciclotizolam | $C_{20}H_{18}BrClN_4S$ | 461.0197 | Cayman Chemical |
| 49 | *N*-Phenethyl-*N*-phenylpropionamide | $C_{17}H_{19}NO$ | 254.1539 | Cayman Chemical |
| 50 | 4-Anilino-1-Boc-piperidine | $C_{16}H_{24}N_2O_2$ | 277.1911 | Cayman Chemical |
| 51 | *N*-Boc Norfentanyl | $C_{19}H_{28}N_2O_3$ | 333.2173 | Cayman Chemical |
| 52 | 3-Acetamidophenol | $C_8H_9NO_2$ | 152.1673 | Sigma Aldrich |
| 53 | *N*-Pyrrolidino Etodesnitazene | $C_{22}H_{27}N_3O$ | 350.2227 | Cayman Chemical |
| 54 | 3,5-Dimethoxyphenol | $C_8H_{10}O_3$ | 155.1673 | Sigma Aldrich |
| 55 | *N*-propyl-*N*-methyl Butylone | $C_{15}H_{21}NO_3$ | 264.1594 | Cayman Chemical |
| 56 | *N*-butyl-*N*-methyl Butylone | $C_{16}H_{23}NO_3$ | 278.1751 | Cayman Chemical |
| 57 | CH-HEXIATA | $C_{22}H_{32}N_2O$ | 341.2587 | Cayman Chemical |
| 58 | CH-PIATA N-pentanoic acid | $C_{21}H_{28}N_2O_3$ | 357.2173 | Cayman Chemical |
| 59 | MDMB-BUTINACA | $C_{19}H_{27}N_3O_3$ | 346.2125 | Cayman Chemical |
| 60 | Tamsulosin | $C_{20}H_{28}N_2O_5S$ | 409.1792 | Cayman Chemical |
| 61 | 4-hydroxy Xylazine | $C_{12}H_{16}N_2OS$ | 237.1056 | Cayman Chemical |
| 62 | 4-Chloromethamphetamine | $C_{10}H_{14}ClN$ | 184.0888 | Cayman Chemical |
| 63 | 4-Chloroamphetamine | $C_9H_{12}ClN$ | 170.0731 | Cayman Chemical |
| 64 | AB-CHMIATA | $C_{22}H_{31}N_3O_2$ | 370.2489 | Cayman Chemical |
| 65 | CHM-FUBIATA | $C_{24}H_{27}FN_2O$ | 379.2180 | Cayman Chemical |
| 66 | CHO-4'Me-5'Br-FUBOXPYRA | $C_{20}H_{22}BrFN_2O_2$ | 421.0921 | Cayman Chemical |
| 67 | 4-Methylaminoantipyrine | $C_{12}H_{15}N_3O$ | 218.2793 | Sigma Aldrich |
| 68 | 4-Formylaminoantipyrine | $C_{12}H_{13}N_3O_2$ | 232.2573 | Sigma Aldrich |
| 69 | 4-Aminoantipyrine | $C_{11}H_{13}N_3O$ | 204.2473 | Sigma Aldrich |
| 70 | Etilefrine | $C_{10}H_{15}NO_2$ | 218.6996 | Cayman Chemical |
| 71 | Meloxicam | $C_{14}H_{13}N_3O_4S_2$ | 352.0420 | Cayman Chemical |
| 72 | *N*-formyl 4-ANPP | $C_{20}H_{24}N_2O$ | 309.1961 | Cayman Chemical |
| 73 | 5F-CUMYL-PINACA N-pentanoic acid | $C_{22}H_{25}N_3O_3$ | 380.1969 | Cayman Chemical |
| 74 | 4'-Chlorodiazepam | $C_{16}H_{12}Cl_2N_2O$ | 319.0399 | Cayman Chemical |
| 75 | Propylhexedrine | $C_{10}H_{21}N$ | 156.1747 | Cayman Chemical |
| 76 | 1cP-MiPLA | $C_{24}H_{29}N_3O_2$ | 392.2333 | Cayman Chemical |
| 77 | Amitraz | $C_{19}H_{23}N_3$ | 294.1965 | Cayman Chemical |

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| 78 | 5-methoxy NiPT | $C_{14}H_{20}N_2O$ | 233.1648 | Cayman Chemical |
| 79 | 2,6-Xylidine | $C_8H_{11}N$ | 122.0964 | Cayman Chemical |
| 80 | 2-methyl-α-PiHP | $C_{17}H_{25}NO$ | 260.2009 | Cayman Chemical |
| 81 | 3-desoxy-3,4-MDPV | $C_{17}H_{23}NO_2$ | 274.1802 | Cayman Chemical |
| 82 | MDMB-5Me-INACA | $C_{16}H_{21}N_3O_3$ | 304.1656 | Cayman Chemical |
| 83 | MDMB-MINACA | $C_{16}H_{21}N_3O_3$ | 304.1656 | Cayman Chemical |
| 84 | MDMB-PICA | $C_{21}H_{30}N_2O_3$ | 359.2329 | Cayman Chemical |
| 85 | MDMB-ICA | $C_{16}H_{20}N_2O_3$ | 289.1547 | Cayman Chemical |
| 86 | 1-(2,6-Xylyl)-2-thiourea | $C_9H_{12}N_2S$ | 181.0794 | Cayman Chemical |
| 87 | Coniine | $C_8H_{17}N$ | 128.1434 | LGC |
| 88 | Bromantane | $C_{16}H_{20}BrN$ | 306.0852 | Cayman Chemical |
| 89 | Methylenedioxynitazene | $C_{21}H_{24}N_4O_4$ | 397.1870 | Cayman Chemical |
| 90 | 9(R)-HHCH | $C_{22}H_{34}O_2$ | 331.2632 | Cayman Chemical |
| 91 | *N*-Phenethylnoroxymorphone | $C_{24}H_{25}NO_4$ | 392.1856 | Cayman Chemical |
| 92 | Varenicline | $C_{13}H_{13}N_3$ | 212.1182 | Cayman Chemical |
| 93 | *N*-methyl Cyclazodone | $C_{13}H_{14}N_2O_2$ | 231.1128 | Cayman Chemical |
| 94 | *N*-desmethyl Rilmazolam | $C_{18}H_{13}Cl_2N_5O$ | 386.0570 | Cayman Chemical |
| 95 | R-6890 | $C_{21}H_{24}ClN_3O$ | 370.1681 | Cayman Chemical |
| 96 | MDMB-BICA | $C_{20}H_{28}N_2O_3$ | 345.2173 | Cayman Chemical |
| 97 | MDMB-CHM7AICA | $C_{22}H_{31}N_3O_3$ | 386.2438 | Cayman Chemical |
| 98 | Tofisopam | $C_{22}H_{26}N_2O_4$ | 383.1965 | Cayman Chemical |
| 99 | Rilmazolam | $C_{19}H_{15}Cl_2N_5O$ | 400.0726 | Cayman Chemical |
| 100 | MMB-4en-PINACA | $C_{19}H_{25}N_3O_3$ | 344.1969 | Cayman Chemical |
| 101 | Tizanidine | $C_9H_8ClN_5S$ | 254.0262 | Cayman Chemical |
| 102 | MMB-ICA | $C_{15}H_{18}N_2O_3$ | 275.1390 | Cayman Chemical |
| 103 | *para*-Fluoro 4-Anilino-1-Boc-Piperidine | $C_{16}H_{23}FN_2O_2$ | 295.1816 | Cayman Chemical |
| 104 | Cyclopropyl norfentanyl | $C_{15}H_{20}N_2O$ | 245.1648 | Cayman Chemical |
| 105 | AH 8533 | $C_{16}H_{23}ClN_2O$ | 295.1572 | Cayman Chemical |
| 106 | Desmethylmoramide | $C_{24}H_{30}N_2O_2$ | 379.2380 | Cayman Chemical |
| 107 | 3-hydroxy Phenazepam | $C_{15}H_{10}BrClN_2O_2$ | 364.9687 | Cayman Chemical |
| 108 | Bretazenil | $C_{19}H_{20}BrN_3O_3$ | 418.0761 | Cayman Chemical |
| 109 | Cloniprazepam | $C_{19}H_{16}ClN_3O_3$ | 370.0953 | Cayman Chemical |
| 110 | Clotizolam | $C_{15}H_{10}Cl_2N_4S$ | 349.0076 | Cayman Chemical |
| 111 | *N*-Desmethylflunitrazepam | $C_{15}H_{10}FN_3O_3$ | 300.0779 | Cayman Chemical |
| 112 | Difludiazepam | $C_{16}H_{11}ClF_2N_2O$ | 321.0601 | Cayman Chemical |
| 113 | Nifoxipam | $C_{15}H_{10}FN_3O_4$ | 316.0728 | Cayman Chemical |

**Table 2:** New drug monographs issued after first detection (https://www.cfsre.org/nps-discovery/monographs).

| # | Drug Name | NPS Class | Sample Type | Date Released |
|---|---|---|---|---|
| 1 | ADB-5'Br-PINACA | Cannabinoid | Small square paper | 5/1/2023 |
| 2 | 4-Methylmethylphenidate | Stimulant | Blood | 6/20/2023 |
| 3 | 25B-NBOH | Hallucinogen | Blood | 6/21/2023 |
| 4 | *N*-Pyrrolidino Protonitazene | Opioid | Brown powder | 6/22/2023 |
| 5 | *N*-Pyrrolidino Metonitazene | Opioid | Tan powder | 6/23/2023 |
| 6 | MDMB-INACA | Cannabinoid | Paper | 6/26/2023 |
| 7 | MDMB-BINACA | Cannabinoid | Blood | 6/27/2023 |
| 8 | CHO-4'Me-5'Br-FUBOXPYRA | Cannabinoid | White powder | 6/28/2023 |
| 9 | 4'-Chlorodiazepam | Benzodiazepine | Liver, gastric fluid | 11/20/2023 |
| 10 | MDMB-5Me-INACA | Cannabinoid | Paper | 11/21/2023 |
| 11 | NMDMSB | Cannabinoid | Red powder | 11/22/2022 |
| 12 | Medetomidine | Miscellaneous | Blood, serum/plasma | 11/27/2023 |
| 13 | MDMB-PICA | Cannabinoid | Blood, urine | 11/28/2023 |
| 14 | MDMB-ICA | Cannabinoid | Blood, urine | 11/28/2023 |
| 15 | Rilmazafone | Benzodiazepine | Pill | 11/29/2023 |
| 16 | *N*-Desethyl Etonitazene | Opioid | Blood | 11/30/2023 |
| 17 | *ortho*-Methylfentanyl | Opioid | Blood | 12/20/2023 |

| | | | | |
|---|---|---|---|---|
| 18 | MMB-4en-PINACA | Cannabinoid | Paper | 6/20/2024 |
| 19 | AB-CHMINACA | Cannabinoid | Paper | 8/19/2024 |
| 20 | Bromantane | Stimulant | Blood | 8/20/2024 |
| 21 | *N*-Phenethyl Noroxymorphone | Opioid | White powder | 8/21/2024 |
| 22 | 3-Methyl-PCP | Hallucinogen | White powder | 8/22/2024 |
| 23 | 5,6-Dichloro Desmethylchlorphine (SR 17018) | Opioid | White powder | 8/23/2024 |
| 24 | 5-Methyl Etodesnitazene | Opioid | White powder | 8/26/2024 |
| 25 | Chloromethcathinone (CMC) | Stimulant | Oral fluid | 8/27/2024 |
| 26 | Desalkylquazepam | Benzodiazepine | Blue powder | 8/28/2024 |
| 27 | Methylenedioxynitazene | Opioid | Tan powder | 8/29/2024 |
| 28 | *ortho*-Methyl boc 4-AP | Opioid | White powder | 8/30/2024 |
| 29 | BTMPS | Miscellaneous | White powder | 9/3/2024 |
| 30 | *N*-Isopropyl Butylone | Stimulant | Off-white crystalline material | 9/4/2024 |
| 31 | MDP2P/PMK | Miscellaneous | Purple crystalline material | 12/13/2024 |
| 32 | 4Cl-MDMB-BINACA | Cannabinoid | Transparent liquid | 12/16/2024 |
| 33 | *N*-Propionitrile Chlorphine | Opioid | White material | 12/17/2024 |
| 34 | Chlorphine | Opioid | White material | 12/17/2024 |
| 35 | HHC | Cannabinoid | Blood | 12/18/2024 |
| 36 | MMMP (Caccure 907) | Miscellaneous | Yellow tablet | 12/19/2024 |
| 37 | *N*-Pyrrolidino Isotonitazene | Opioid | Blood | 12/20/2024 |

### *4.2 Objective 2: NPS Monitoring*

During the project period, our laboratory received 785 forensic toxicology specimens from authentic casework (e.g., medicolegal death investigations (MDI), driving under the influence of drugs (DUID) investigations, clinical intoxications, overdose events, outbreaks, etc.). Our laboratory also partnered with NMS Labs and tested 7,749 sample extracts for the presence of NPS during the project period. From the sample populations described, 79 different NPS were identified in 2023 with 3,601 total identifications and 75 NPS were identified in Q1-Q3 2024 with 2,221 total identifications (**Table 3**). Q4 2024 data was still being consolidated at the time of writing this report. Using data acquired from NPS monitoring efforts, 12 trend reports depicting NPS positivity (**Figures 1-4**), five public alerts (**Table 4**), and one Year in Review report (**Figure 5**) were issued. Four additional trend reports for NPS identified in Q4 2024, as well as the 2024 Year in Review report, will be released in January 2025.

**Table 3:** Total NPS identifications in 2023 and 2024 (excluding Q4 2024).

| 2023 | | | 2024 | | |
|---|---|---|---|---|---|
| **Drug Name** | **NPS Subclass** | **#** | **Drug Name** | **NPS Subclass** | **#** |
| 4'-Chlorodiazepam | Benzodiazepine | 1 | 4'Cl-Deschloroalprazolam | Benzodiazpine | 4 |
| Adinazolam | Benzodiazepine | 1 | Adinazolam | Benzodiazpine | 4 |
| Bromazepam | Benzodiazepine | 2 | Bromazepam | Benzodiazpine | 3 |
| Bromazolam | Benzodiazepine | 926 | Bromazolam | Benzodiazpine | 905 |
| Clonazolam | Benzodiazepine | 5 | Clonazolam | Benzodiazpine | 20 |
| Delorazepam | Benzodiazepine | 3 | Desalkylflurazepam | Benzodiazpine | 4 |
| Desalkylflurazepam | Benzodiazepine | 8 | Desalkylgidazepam | Benzodiazpine | 52 |
| Desalkylgidazepam | Benzodiazepine | 45 | Desalkylquazepam | Benzodiazpine | 1 |
| Deschloroetizolam | Benzodiazepine | 4 | Deschloroetizolam | Benzodiazpine | 3 |
| Etizolam | Benzodiazepine | 44 | Etizolam | Benzodiazpine | 12 |
| Flualprazolam | Benzodiazepine | 47 | Flualprazolam | Benzodiazpine | 30 |
| Flubromazepam | Benzodiazepine | 67 | Flubromazepam | Benzodiazpine | 43 |
| Flubromazolam | Benzodiazepine | 8 | Flubromazolam | Benzodiazpine | 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Metizolam | Benzodiazepine | 1 | | Phenazolam | Benzodiazpine | 10 |
| Pyrazolam | Benzodiazepine | 5 | | 4CN-CUMYL BINACA | Cannabinoid | 1 |
| Rilmazafone | Benzodiazepine | 2 | | 5F-MDMB-PICA | Cannabinoid | 2 |
| 4F-MDMB-BICA | Cannabinoid | 3 | | 5F-MDMB-PINACA (5F-ADB) | Cannabinoid | 28 |
| 4F-MDMB-BINACA | Cannabinoid | 1 | | AB-CHMINACA | Cannabinoid | 11 |
| 5F-ADB | Cannabinoid | 3 | | ADB-4en-PINACA | Cannabinoid | 14 |
| 5F-MDMB-PICA | Cannabinoid | 1 | | ADB-BINACA | Cannabinoid | 9 |
| ADB-4en-PINACA | Cannabinoid | 2 | | ADB-HEXINACA | Cannabinoid | 1 |
| ADB-5'Br-PINACA | Cannabinoid | 1 | | ADB-INACA | Cannabinoid | 4 |
| ADB-5'Br-BINACA | Cannabinoid | 1 | | CH-FUBIATA | Cannabinoid | 1 |
| ADB-BINACA | Cannabinoid | 13 | | CHO-4'Me-5'Br-FUBOXPYRA | Cannabinoid | 1 |
| ADB-INACA | Cannabinoid | 1 | | MDMB-4en-PINACA | Cannabinoid | 102 |
| CHO-4'Me-5'Br-FUBOXPYRA | Cannabinoid | 1 | | MDMB-5Br-INACA | Cannabinoid | 1 |
| CH-PIATA | Cannabinoid | 1 | | MDMB-5Me-INACA | Cannabinoid | 2 |
| MDMB-4en-PINACA | Cannabinoid | 59 | | MDMB-BINACA | Cannabinoid | 14 |
| MDMB-5Me-INACA | Cannabinoid | 1 | | MDMB-ICA | Cannabinoid | 1 |
| MDMB-BINACA | Cannabinoid | 2 | | MDMB-INACA | Cannabinoid | 26 |
| MDMB-ICA | Cannabinoid | 1 | | MMB-4en-PINACA | Cannabinoid | 1 |
| MDMB-INACA | Cannabinoid | 10 | | 2C-B | Hallucinogen | 7 |
| MDMB-PICA | Cannabinoid | 1 | | 2C-C | Hallucinogen | 2 |
| NMDMSB | Cannabinoid | 1 | | 2F-2oxo-PCE | Hallucinogen | 104 |
| 25B-NBOH | Hallucinogen | 1 | | 3-Methyl-PCP | Hallucinogen | 2 |
| 2C-B | Hallucinogen | 4 | | Deoxymethoxetamine (DMXE) | Hallucinogen | 1 |
| 2F-Deschloroketamine | Hallucinogen | 2 | | DOB (4-bromo-2,5-DMA) | Hallucinogen | 4 |
| 4-MeO-PCP | Hallucinogen | 1 | | MeO-DMT | Hallucinogen | 1 |
| Deschloroketamine | Hallucinogen | 2 | | MeO-PCP | Hallucinogen | 2 |
| 2F-2oxo-PCE | Hallucinogen | 31 | | 5-Methyl Etodesnitazene | Opioid | 4 |
| MBZP | Hallucinogen | 1 | | AP-238 | Opioid | 1 |
| *N*-ethyl Deschloroketamine | Hallucinogen | 1 | | Brorphine | Opioid | 7 |
| Furanyl UF-17 | Miscellaneous | 2 | | Carfentanil | Opioid | 52 |
| Medetomidine | Miscellaneous | 6 | | Chlorphine | Opioid | 1 |
| Acetyl Fentanyl | Opioid | 135 | | Dipyanone | Opioid | 1 |
| Bromofentanyl | Opioid | 5 | | Etodesnitazene | Opioid | 8 |
| Brorphine | Opioid | 4 | | Isotonitazene | Opioid | 3 |
| Carfentanil | Opioid | 6 | | Methylenedioxynitazene | Opioid | 3 |
| Dipyanone | Opioid | 1 | | Methyltetrahydrofuranylfentanyl | Opioid | 8 |
| Etodesnitazene | Opioid | 2 | | Metodesnitazene | Opioid | 2 |
| Fluorofentanyl | Opioid | 1539 | | Metonitazene | Opioid | 89 |
| Isotonitazene | Opioid | 7 | | *N*-Desethyl Etonitazene | Opioid | 20 |
| Metonitazene | Opioid | 53 | | *N*-Desethyl Isotonitazene | Opioid | 13 |
| *N*-Desethyl Etonitazene | Opioid | 2 | | *N*-Propionitrile Chlorphine | Opioid | 1 |
| *N*-Desethyl Isotonitazene | Opioid | 6 | | *N*-Pyrrolidino Etonitazene | Opioid | 48 |
| *N*-Pyrrolidino Etonitazene | Opioid | 3 | | *N*-Pyrrolidino Metonitazene | Opioid | 7 |
| *N*-Pyrrolidino Metonitazene | Opioid | 4 | | *N*-Pyrrolidino Protonitazene | Opioid | 28 |
| *N*-Pyrrolidino Protonitazene | Opioid | 15 | | *ortho*-Methylfentanyl | Opioid | 207 |
| *ortho*-Methylfentanyl | Opioid | 1 | | Protonitazene | Opioid | 80 |
| *para*-Fluoro Valeryl Fentanyl | Opioid | 1 | | *Alpha*-PHP / *Alpha*-PiHP | Stimulant | 5 |
| *para*-Methyl THF-fentanyl | Opioid | 3 | | Chloromethcathinone | Stimulant | 5 |
| Protonitazene | Opioid | 34 | | Chloromethcathinone | Stimulant | 1 |
| Tetrahydrofuran Fentanyl | Opioid | 1 | | Deschloroketamine | Stimulant | 1 |
| Tianeptine | Opioid | 2 | | Eutylone | Stimulant | 2 |
| Valerylfentanyl | Opioid | 4 | | MDPHP | Stimulant | 1 |
| 3-Chlorocathinone | Stimulant | 1 | | MDPHP / MDPiHP | Stimulant | 1 |
| 4F-*alpha*-PHP | Stimulant | 1 | | Methylmethcathinone | Stimulant | 15 |
| 4F-Methylphenidate | Stimulant | 1 | | Methylone | Stimulant | 1 |
| 4-Methylmethylphenidate | Stimulant | 1 | | *N,N*-Dimethylpentylone | Stimulant | 111 |
| *Alpha*-PHP / *Alpha*-PiHP | Stimulant | 22 | | *N*-Cyclohexyl Butylone | Stimulant | 1 |
| *Alpha*-PVP | Stimulant | 1 | | *N*-Cyclohexyl Methylone | Stimulant | 4 |
| *N,N*-Dimethylpentylone | Stimulant | 262 | | *N*-Cyclohexyl Methylone | Stimulant | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ethylone | Stimulant | 1 | | *N*-Ethyl Pentylone | Stimulant | 3 |
| Eutylone | Stimulant | 9 | | *N*-Isopropyl Butylone | Stimulant | 2 |
| Mephedrone | Stimulant | 2 | | Pentylone | Stimulant | 47 |
| Methylenedioxy-PV8 | Stimulant | 1 | | | | |
| Methylone | Stimulant | 1 | | | | |
| *N*-Cyclohexyl Methylone | Stimulant | 1 | | | | |
| Pentylone | Stimulant | 146 | | | | |

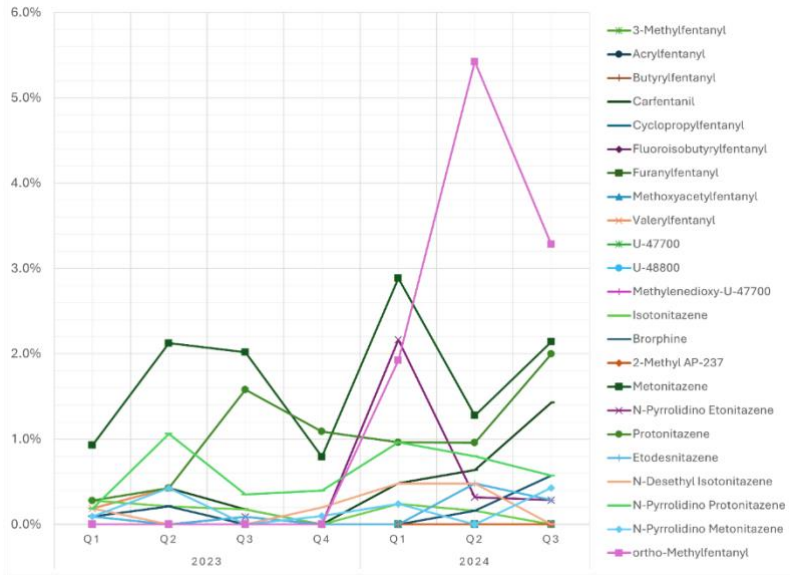


**Figure 1:** Positivity plot showing NPS opioids in toxicology samples analyzed by LC-QTOF-MS between Q1 2023 and Q3 2024 (https://www.cfsre.org/nps-discovery/trend-reports).

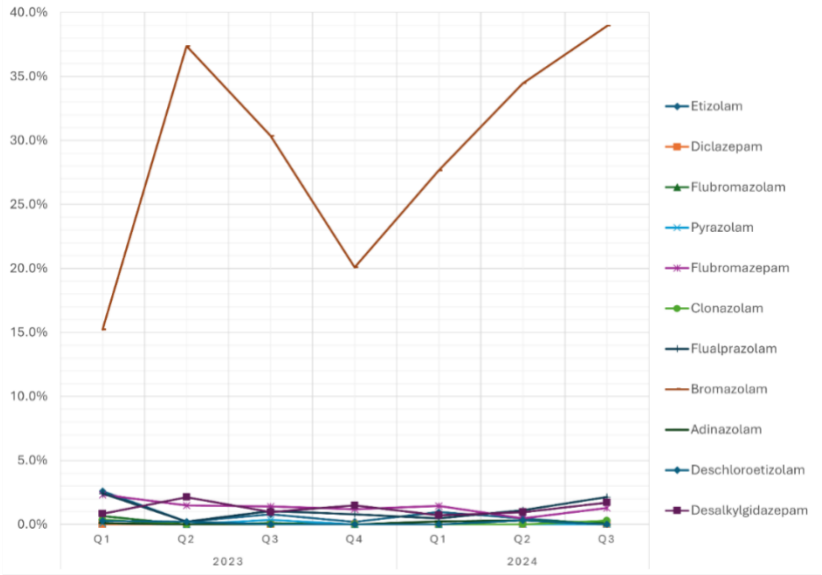


**Figure 2:** Positivity plot showing NPS benzodiazepines in toxicology samples analyzed by LC-QTOF-MS between Q1 2023 and Q3 2024 (https://www.cfsre.org/nps-discovery/trend-reports).

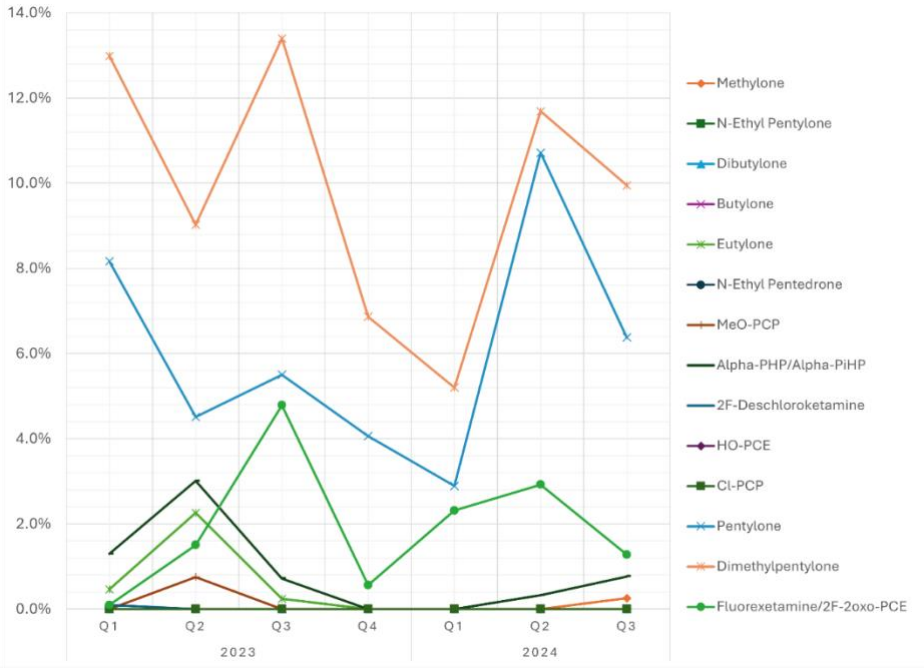


**Figure 3:** Positivity plot showing NPS stimulants and hallucinogens in toxicology samples analyzed by LC-QTOF-MS between Q1 2023 and Q3 2024 (https://www.cfsre.org/nps-discovery/trend-reports).

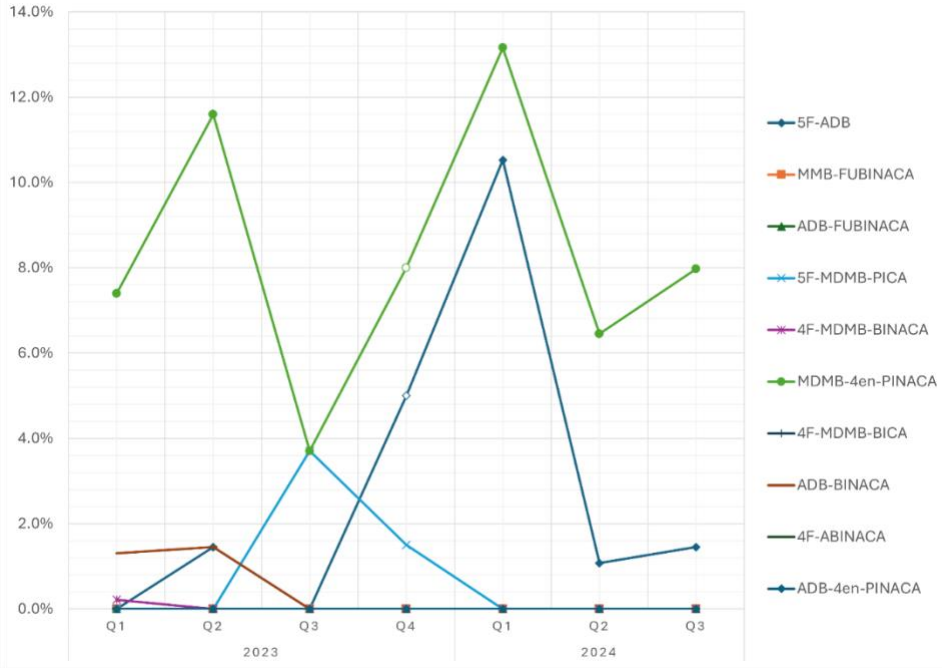


**Figure 4:** Positivity plot showing synthetic cannabinoids in toxicology samples analyzed by LC-QTOF-MS between Q1 2023 and Q3 2024 (https://www.cfsre.org/nps-discovery/trend-reports).

**Table 4:** Public alerts issued for NPS of concern to public health and safety (https://www.cfsre.org/nps-discovery/public-alerts).

| # | Drug Name | Date Released | Reason for Alert |
|---|---|---|---|
| 1 | *N*-Desethyl Isotonitazene | 01/23/2023 | 7 drug materials |
| 2 | *N*-Pyrrolidino Protonitazene | 08/29/2023 | 20 fatal overdoses |
| 3 | Medetomidine | 05/20/2024 | 2 mass overdose outbreaks and appearance in drug materials |
| 4 | 2F-2-oxo-PCE | 05/30/2024 | 35 fatal and non-fatal overdoses and 20 drug materials |
| 5 | *ortho*-Methylfentanyl | 12/03/2024 | >200 fatal overdoses |

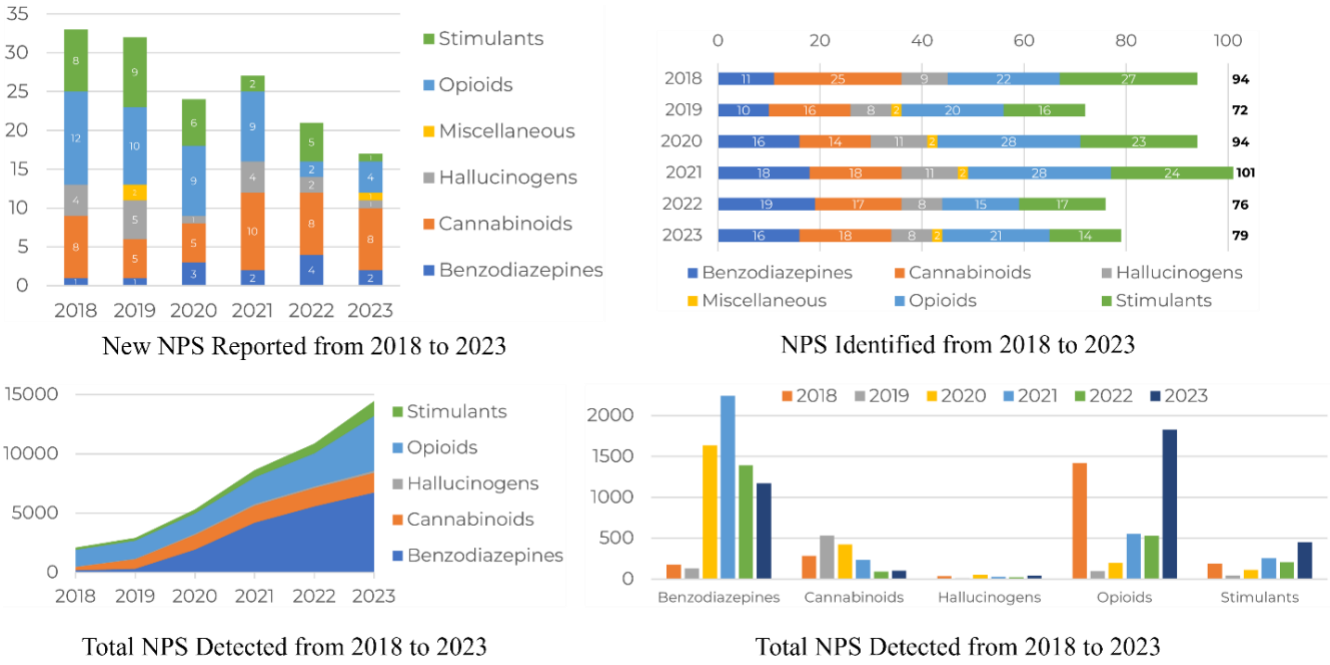


**Figure 5:** Figures from 2023 *Year in Review* depicting the emergence of NPS by subclass (https://www.cfsre.org/nps-discovery/trend-reports).

*4.3 Objective 3: NPS Metabolism*

The primary metabolites of one benzodiazepine (rilmazolam), one synthetic cannabinoid (CHO-4'Me-5'Br-FUBOXPYRA), and one miscellaneous NPS (medetomidine) were investigated through *in vitro* experiments using LC-QTOF-MS. These NPS were chosen due to the suspected potential increase in intoxications and overdoses from partnering laboratories and no metabolism data in the literature review. The process allowed our laboratory to include the metabolites of these NPS in our scope for surveillance. The primary biotransformations for each NPS were determined through analysis of authentic biological specimens (**Table 5**). Proposed metabolic schemes based on *in vitro* analyses are shown in **Figures 6-8**.

**Table 5:** Primary biotransformations identified from *in vitro* metabolism experiments alongside resulting data acquired by LC-QTOF-MS.

| Drug | Biotransformation | Formula | [M+H] (Da) | Mass Error (ppm) | Retention Time (min) |
|---|---|---|---|---|---|
| Rilmazolam | ***Parent*** | ***$C_{19}H_{15}Cl_2N_5O$*** | ***399.0653*** | ***-1.0*** | ***7.94*** |
| | *N*-demethylation | $C_{18}H_{13}Cl_2N_5O$ | 386.0497 | -0.6 | 7.53 |
| | *N,N*-didemethylation | $C_{17}H_{11}Cl_2N_5O$ | 371.0340 | 2.2 | 7.28 |
| | Carboxylation | $C_{17}H_{10}Cl_2N_4O_2$ | 372.0253 | 1.3 | 7.05 |
| CHO-4'Me-5'Br-FUBOXPYRA | ***Parent*** | ***$C_{20}H_{22}BrFN_2O_2$*** | ***420.0800*** | ***-0.1*** | ***8.82*** |
| | Hydroxylation | $C_{20}H_{22}BrFN_2O_3$ | 436.08 | -0.4 | 7.08 |
| | Hydroxylation | $C_{20}H_{22}BrFN_2O_3$ | 436.08 | -0.3 | 7.72 |
| | Hydroxylation | $C_{20}H_{22}BrFN_2O_3$ | 436.08 | -3.5 | 7.43 |
| | Hydroxylation | $C_{20}H_{22}BrFN_2O_3$ | 436.08 | -3.4 | 7.25 |
| Medetomidine | ***Parent*** | ***$C_{13}H_{16}N_2$*** | ***200.13*** | ***0.6*** | ***5.69*** |
| | Hydroxylation | $C_{13}H_{16}N_2O$ | 216.13 | 1.2 | 3.99 |
| | Hydroxylation | $C_{13}H_{16}N_2O$ | 216.13 | -0.3 | 7.01 |
| | Hydroxylation | $C_{13}H_{16}N_2O$ | 216.13 | 0 | 4.56 |
| | Hydroxylation | $C_{13}H_{16}N_2O$ | 216.13 | 0.4 | 4.69 |

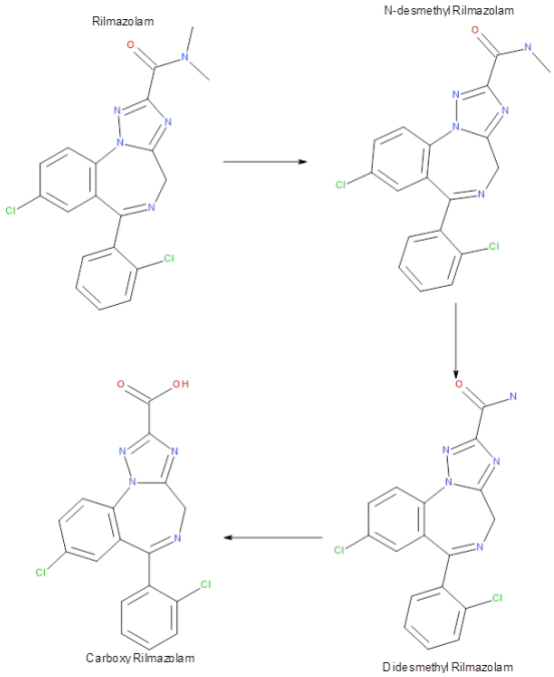


**Figure 6:** Proposed metabolism scheme for rilmazolam (NPS benzodiazepine).

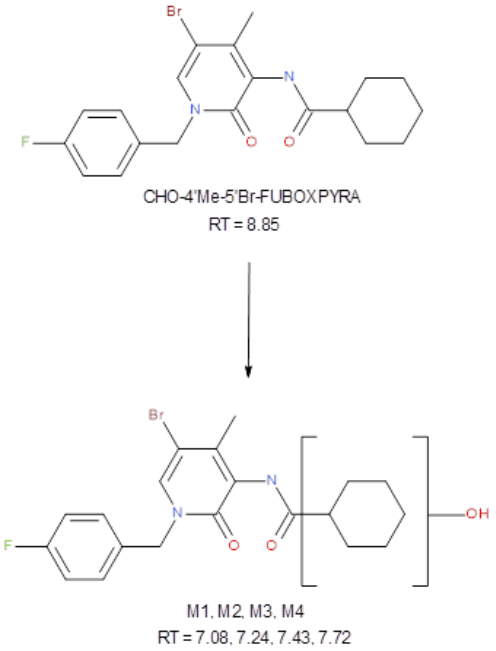


**Figure 7:** Proposed metabolism scheme for CHO-4'Me-5'Br-FUBOXPYRA (syntheticcannabinoid).

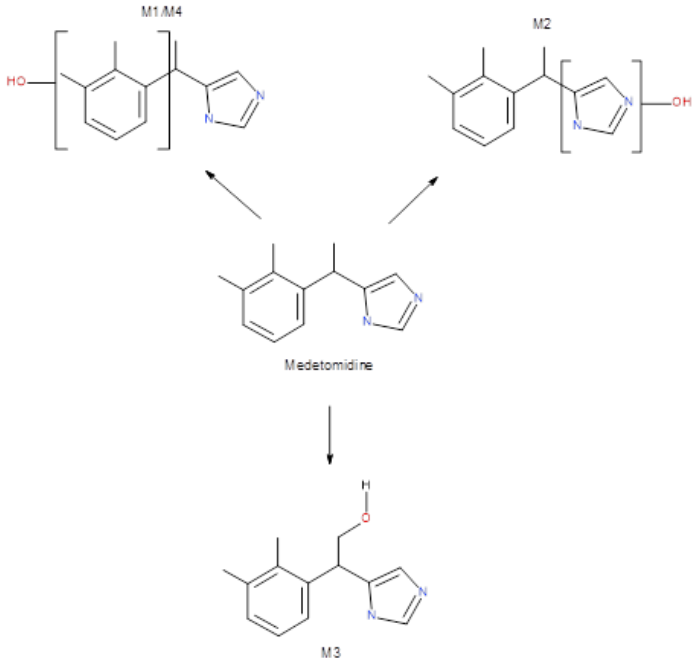


**Figure 8:** Proposed metabolism scheme for medetomidine (miscellaneous/adulterant).

*4.4 Objective 4: NPS Confirmation*

Quantitative confirmations were completed for dipyanone, synthetic cathinones (*N,N*-dimethylpentylone, pentylone), and nitazene analogues (etodesnitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino metonitazene, and *N*-pyrrolidino protonitazene). The application of these methods to authentic samples provided the forensic community with previously unknown reference concentration data on these NPS. All methods were successfully developed and validated according to ASB Standard 036 and 054 and applied to authentic biological samples. The quantitative methods were both standard addition (dipyanone and nitazene analogues) and external calibration (synthetic cathinones) methods. Reference data acquired from these studies are shown in **Tables 6-8**.

**Table 6:** Reference concentration data for *N,N*-dimethylpentylone in fatal overdoses compared to eutylone and *N*-ethyl pentylone (https://academic.oup.com/jat/article/47/8/753/7199914).

| [ng/mL] | Eutylone (n=67) | *N*-Ethyl Pentylone (n=19) | *N,N*-Dimethylpentylone (n=18) |
|---|---|---|---|
| Mean (±SD) | 1,020 (±2,242) | 385 (±381) | 277 (±283) |
| Median | 110 | 210 | 145 |
| Range | 1.2–11,000 | 12–1,200 | 3.3–970 |

**Table 7:** Reference concentration data and case information for dipyanone in one fatal overdose (https://link.springer.com/article/10.1007/s00216-023-04722-7).

| Dipyanone Fatality | |
|---|---|
| **Collection Date** | 9/6/2022 |
| **State, Country** | Washington, USA |
| **Sex, Age** | Male, 38 |
| **Matrix** | Femoral blood |
| **Case History** | Found deceased in home during a welfare check. EMS confirmed without intervention. White powder residue and tooter found in home along with an empty Rx bottle (ketamine). Presumptive using screen positive for methadone, tramadol, and methamphetamine. |
| **[Dipyanone]** | 370 ng/mL |
| **Additional blood toxicology results (ng/mL)** | 2-Methyl AP-237 **(24)**, 8-aminoclonazolam **(7.5)**, flualprazolam **(5.7)**, delorazepam **(6.2)**, bupropion **(100)**, hydroxybupropion **(250)**, O-desmethyltramadol **(560)**, chlorpheniramine **(48)**, dextrorphan/levanorphanol **(33)**, dextro/levo methorphan **(450)**, citalopram **(490)**, pseudoephedrine **(180)** |

**Table 8:** Reference concentration data for novel nitazene analogues in fatal overdoses (https://link.springer.com/article/10.1007/s00204-024-03774-7).

| Drug | Qualitative (N) | Quantitative (N) | Mean (±SD) (ng/mL) | Median (ng/mL) | Range (ng/mL) |
|---|---|---|---|---|---|
| Etodesnitazene | 26 | 15 | 25 ± 36 | 4 | 0.1–120 |
| *N*-Pyrrolidino Protonitazene | 39 | 26 | 8 ± 17 | 1.2 | 0.3–55 |
| *N*-Pyrrolidino Metonitazene | 15 | 11 | 3 ± 7 | 0.47 | 0.2–26 |
| *N*-Desethyl Isotonitazene | 16 | 9 | 4 ± 2 | 3.4 | 0.82–8.3 |

*4.5 Objective 5: NPS Toolkits*

NPS toolkits are a consolidation of all data and information gathered about a particular NPS or NPS class (https://www.cfsre.org/nps-discovery/). NPS toolkits contain information regarding analytical and instrument data (e.g., mass spectra, retention time, analytical methods), drug intelligence, drug trends and combinations, geographical and demographic information, public alerts, and metabolism. New NPS toolkits are pending finalization (https://www.cfsre.org/nps-discovery/analytical-toolkits).

## 5. Applicability to Criminal Justice

This study had high impact on the criminal justice system as it built on techniques and approaches previously used among forensic science laboratories and in previous NIJ-funded research. As NPS continue to appear in illicit drug markets and cause overdoses, outbreaks, and fatalities, the criminal justice system must rely heavily on the information gathered by forensic scientists and chemists to prosecute crimes involving drugs and develop legislation. Identifying NPS in forensic casework and linking to adverse effects or toxicity informs drug policy and as the Drug Enforcement Administration (DEA) moves to schedule emerging drugs, the work provided by our laboratory directly impacted drug legislation and scheduling actions (**Table 9**). Improved NPS surveillance, monitoring, and characterization allowed for an increased understanding of NPS in laboratories and criminal justice agencies, which in turn had significant impact to public health and safety.

**Table 9:** Drug Scheduling Actions Impacted by CFSRE NPS Data.

| Drug Name | Scheduling Action | Date | Link |
|---|---|---|---|
| Brorphine | Final Order: Placement in Schedule I | 03/06/2023 | https://www.federalregister.gov/documents/2023/03/06/2023-04364/schedules-of-controlled-substances-placement-of-brorphine-in-schedule-i |
| Etizolam | Temporary Scheduling Order: Placement in Schedule I | 07/26/2023 | https://www.federalregister.gov/documents/2023/07/26/2023-15748/schedules-of-controlled-substances-temporary-placement-of-etizolam-flualprazolam-clonazolam |
| Flualprazolam | | | |
| Clonazolam | | | |
| Flubromazolam | | | |
| Diclazepam | | | |
| Metonitazene | Final Order: Placement in Schedule I | 08/18/2023 | https://www.federalregister.gov/documents/2023/08/18/2023-17778/schedules-of-controlled-substances-placement-of-metonitazene-in-schedule-i |
| MDMB-4en-PINACA | Temporary Scheduling Order: Placement in Schedule I | 12/12/2023 | https://www.federalregister.gov/documents/2023/12/12/2023-27243/schedules-of-controlled-substances-temporary-placement-of-mdmb-4en-pinaca-4f-mdmb-butica |
| 4F-MDMB-BUTICA | | | |
| ADB-4en-PINACA | | | |
| CUMYL-PEGACLONE | | | |
| 5F-EDMB-PICA | | | |
| MMB-FUBICA | | | |
| 2-Methyl AP-237 | Final Order: Placement in Schedule I | 03/15/2024 | https://www.federalregister.gov/documents/2024/03/15/2024-05543/schedules-of-controlled-substances-placement-of-2-methyl-ap-237-in-schedule-i |
| *N*-Desethyl Isotonitazene | Temporary Scheduling Order: Placement in Schedule I | 07/29/2024 | https://www.federalregister.gov/documents/2024/07/29/2024-16391/schedules-of-controlled-substances-temporary-placement-of-n-desethyl-isotonitazene-and-n-piperidinyl |
| *N*-Piperidinyl Etonitazene | | | |

## PRODUCTS

### 1. List of All Scholarly Products

The data acquired from the surveillance, monitoring, characterization and dissemination of information regarding NPS was consolidated into publications and reports for knowledge transfer to stakeholders (e.g., forensic toxicologists, forensic chemists, medical examiners, coroners, police departments, clinicians, and federal entities). During this project period, more than 15 peer-reviewed publications were published (or are currently under review/pending publication), and 33 conference presentations were presented. All data gathered from this project are housed at https://www.cfsre.org/nps-discovery.

*1.1 Peer Reviewed Publications*

- Amy Nham, John N. Le, Shawn A. Thomas, Kimberly Gressick, Emily N. Ussery, Jean Y. Ko, R. Matt Gladden, Christina Mikosz, Joshua G. Schier, Alana Vivolo-Kantor, Maria Fiorillo, McKenna McMaster, Darlene Nolasco Magana, Livia Verklan-McInnes, Michael Wahl, Taylor Wood, Axel Adams, Alex Krotulski, Jordan Trecki, Ross Ellison, Roy Gerona, Ponni Arunkumar, Mojde Mir, Leslie M. Wise, Emma Betancourt, Kathleen Monty, Jhoanna Gulmatico, Angie Pojas, Ruchi Fitzgerald, Miao Hua. Medetomidine Overdose Outbreak — Chicago, Illinois, May 2024. *Morbidity and Mortality Weekly Report (MMWR)*. 2025. [Under Review]
- Christopher J. Counts, Anthony V. Spadaro, Trevor A. Cerbini, Alex J. Krotulski, Sara E. Walton, Howard A. Greller, Lewis S. Nelson, Bruce E. Ruck, Oliver Hung, Barry Logan, Diane P. Calello. An Outbreak of Synthetic Cannabinoid-Adulterated Tianeptine Products in New Jersey – Case Series. *Journal of Medical Toxicology*. 2025. [Under Review]
- Andrew Miller, Alex Krotulski, Sara Walton, Anna Dulaney, Aaron Frolichstein, Haley Dusek, Christine Murphy. Kratom Cardiotoxicity: A Case Report of Reversible Brugada Pattern and QTc Prolongation. *JACC Case Reports*. 2024. [Accepted]
- David Kuai, Liz Eneida Rivera Blanco, Alex Krotulski, Sara Walton, Max Denn, Byron Kelly, Emily Kiernan, Alaina Steck, Joseph Carpenter. Identification and Health Risks of an Emerging Means of Drug Use in Correctional Facilities. *JAMA Open Network*. 2024. https://doi.org/10.1001/jamanetworkopen.2024.51951
- Richard Bade, Denice van Herwerden, Nikolaos Rousis, Sangeet Adhikari, Darren Allen, Christine Baduel, Lubertus Bijlsma, Tim Boogaerts, Dan Burgard, Andrew Chappell, Erin M Driver, Fernando Fabriz Sodre, Despo Fatta-Kassinos, Emma Gracia-Lor, Elisa Gracia-Marín, Rolf U Halden, Ester Heath, Emma Jaunay, Alex Krotulski, Foon Yin Lai, Arndís Sue Ching Löve, Jake W O'Brien, Jeong-Eun Oh, Daniel Pasin, Marco Pineda Castro, Magda Psichoudaki, Noelia Salgueiro-Gonzalez, Cezar Silvino Gomes, Bikram Subedi, Kevin V Thomas, Nikolaos Thomaidis, Degao Wang, Viviane Yargeau, Saer Samanipour, Jochen Mueller. Workflow to facilitate the detection of new psychoactive substances and drugs of abuse in influent urban wastewater. *Journal of Hazardous Materials*. 2024. https://doi.org/10.1016/j.jhazmat.2024.133955
- Joseph J. Palamar, Alex J. Krotulski. Medetomidine Infiltrates the US Illicit Opioid Market. *JAMA*. 2024. https://doi.org/10.1001/jama.2024.15992

- Lorenzo Gitto, Tracy Wadsworth, Daniel Isenschmid, Alex J. Krotulski, Barry K. Logan, PhD, Ilaria Tarozzi, Ponni Arunkumar. MDMB-4en-PINACA–Related Deaths in Cook County Jail. *The American Journal of Forensic Medicine and Pathology*. 2024. https://doi.org/10.1097/PAF.0000000000000966
- Liz Eneida Rivera Blanco, David Kuai, Nicholas Titelbaum, Babar Fiza, David Reehl, Zakaa Hassan, Nader Dbouk, Alex J. Krotulski, Barry K. Logan, Sara E. Walton, Irene Liu, Michael Yu, Joseph Carpenter. Death from bongkrekic acid toxicity: first report in North America. *Toxicology Communications*. 2024. https://doi.org/10.1080/24734306.2024.2377524
- Liam M. De Vrieze, Sara E. Walton, Eline Pottie, Donna Papsun, Barry K. Logan, Alex J. Krotulski, Christophe P. Stove, Marthe M. Vandeputte. In vitro structure–activity relationships and forensic case series of emerging 2-benzylbenzimidazole 'nitazene' opioids. *Archives of Toxicology*. 2024. https://doi.org/10.1007/s00204-024-03774-7
- Eduardo G. de Campos, David G. Farrar, Alex J. Krotulski. Identification of ADB-5'Br-BINACA in plant material and analytical characterization using GC-MS, LC-QTOF-MS, NMR and ATR-FTIR. *Journal of Pharmaceutical and Biomedical Analysis*. 2024. https://doi.org/10.1016/j.jpba.2024.116254
- Christopher J. Counts, Anthony V. Spadaro, Trevor A. Cerbini, Alex J. Krotulski, Howard A. Greller, Lewis S. Nelson, Bruce E. Ruck, Diane P. Calello. Notes from the Field: Cluster of Severe Illness from Neptune's Fix Tianeptine Linked to Synthetic Cannabinoids — New Jersey, June–November 2023. *Morbidity and Mortality Weekly Report (MMWR)*. 2024. http://dx.doi.org/10.15585/mmwr.mm7304a5
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- Melissa F. Fogarty, Alex J. Krotulski, Donna M. Papsun, Sara E. Walton, Michael Lamb, Michael T. Truver, Chris W. Chronister, Bruce A. Goldberger, Barry K. Logan. N, N-Dimethylpentylone (Dipentylone)—A New Synthetic Cathinone Identified in a Postmortem Forensic Toxicology Case Series. *Journal of Analytical Toxicology*. 2023. https://doi.org/10.1093/jat/bkad037
- Marthe M. Vandeputte, Sara E. Walton, Sarah A. Shuda, Donna M. Papsun, Alex J. Krotulski, Christophe P. Stove. Detection, chemical analysis, and pharmacological characterization of dipyanone and other new synthetic opioids related to prescription

drugs. *Analytical and Bioanalytical Chemistry*. 2023. https://doi.org/10.1007/s00216-023-04722-7

*1.2 Book Chapters, Theses, Conference Proceedings, etc.*

- See below (*2.1 Conference Presentations*)

*1.3 Technologies Developed (Patents, Prototypes, etc.)*

- N/A

*1.4 Software, Databases, Other Products*

- N/A

*1.5 Archived Research Data*

- CFSRE's NPS Discovery – https://www.cfsre.org/nps-discovery
- Monographs – https://www.cfsre.org/nps-discovery/monographs
- Trend Reports & Year in Review – https://www.cfsre.org/nps-discovery/trend-reports
- Public Alerts – https://www.cfsre.org/nps-discovery/public-alerts
- Scope Recommendations – https://www.cfsre.org/nps-discovery/scope-recommendations
- Toolkits – https://www.cfsre.org/nps-discovery/analytical-toolkits
- Publications – https://www.cfsre.org/resources/publications
- Presentations – https://www.cfsre.org/resources/presentations

**2. List of All Dissemination Activities (with DOIs or other durable links)**

Dissemination of results was key to the success of this research as the information directly impacts and supports law enforcement, emergency, medical, and laboratory personnel. Peer-reviewed publications, email alerts, presentations at webinars or conference proceedings, and reports (e.g., public alerts, new drug monographs, trend reports) were disseminated as a main objective of this research. This allowed for easy and complete access to drug prevalence and emergence information. Sixteen workshop presentations and four podcasts were presented during the project period.

*2.1 Conference presentations*

- Emerging Drug Trends. Alex J. Krotulski*. R3 Harm Reduction/Naloxone Learning Collaborative, Virtual Presentation, December 2024.
- Synthetic Cannabinoid – A Changing Landscape in the United States. Alex J. Krotulski*. University of Mississippi Medical Center (UMMC) Speaker Series, Virtual Presentation, December 2024.
- NPS Trends in North America. Alex J. Krotulski*. 2024 Novel Synthetic Drug Threat Symposium, Platform Presentation, November 2024.

- Novel Synthetic Opioids & Cannabinoids. Alex J. Krotulski*. 2024 Novel Synthetic Drug Threat Symposium, Platform Presentation, November 2024.
- An Open Access Drug Early Warning System to Assess the Dynamic U.S. Recreational Drug Supply. Alex J. Krotulski*. Philadelphia City-Wide Medical Toxicology Day, Virtual Presentation, October 2024.
- A Collaborative Approach to New Drug Identification in an Emerging Drug Early Warning System. Donna M. Papsun*, Alex J. Krotulski*. Lisbon Addictions, Platform Presentation, October 2024.
- North American Drug Early Warning System Tracking and Combatting New Synthetic Opioids (And Other NPS!). Alex J. Krotulski*. UKIAFT 2024 Meeting, Virtual Presentation, October 2024.
- Forecasting Future NPS Market Challenges and Impacts Based on U.S. Experiences. Alex J. Krotulski*. Drug early warning systems: Resilience for the future, European Union Drugs Agency (EUDA) Meeting, Platform Presentation, October 2024.
- Non-Targeted LC-QTOF-MS Analysis and Targeted LC-QQQ-MS Analysis of Novel Psychoactive Substances. Sara E. Walton*. NMS Labs Scientific Engagement Series, 2024.
- LC-QTOF-MS and LC-QQQ-MS Analysis of Novel Psychoactive Substances. Sara Walton*. NPS Discovery: Novel Synthetic Drug Threats Symposium, Philadelphia, PA, November 2024.
- Prevalence and Quantitative Analysis of 2F-2-oxo-PCE in Toxicology Specimens Collected in the United States. Alyssa Reyes*, Sara Walton, Lindsey Domonoski, Alex Krotulski, Barry Logan. Society of Forensic Toxicologists, St. Louis, MO, 2024.
- Emergence of Medetomidine as an Opioid Adulterant Encountered with Fentanyl, Xylazine, and Other Substances. Barry Logan*, Sara Walton, Brianna Stang, Alyssa Reyes, Alex Krotulski. Society of Forensic Toxicologists, St. Louis, MO, 2024.
- Emergence of ortho-Methylfentanyl in Medicolegal Death Investigation Cases from North America. Sara Walton*, Brianna Stang, Alex Krotulski, Donna Papsun, Brianna Peterson, Aaron Shapiro, Sandrine Merette, Barry Logan. Society of Forensic Toxicologists, St. Louis, MO, 2024.
- A "Tail" of Two Cities – When Old Synthetic Cannabinoids Become New Again. Alex Krotulski*, Sara Walton, Josh DeBord, Dan Anderson, Donna Papsun, Barry Logan. Society of Forensic Toxicologists, St. Louis, MO, 2024.
- Understanding the Current State of Synthetic Cannabinoids – Newly Emerging Drugs, Changing Drug Market Trends, and Implications in Jail Deaths. Alex J. Krotulski*, Sara E. Walton, Max T. Denn, Joshua S. DeBord, Donna M. Papsun, Barry K. Logan. National Association of Medical Examiners Annual Meeting, Oral Presentation, September 2024.
- Novel Psychoactive Substances – A Multi-Pronged Approach To Detection, Safety, and Risk Mitigation. Alex J. Krotulski*. NIJ National Research Conference, Oral Presentation, Panel Discussion, September 2024.
- CFSRE's NPS Discovery Program. Alex J. Krotulski*. Pennsylvania Interagency Substance Use Response Team (ISURT) Meeting, Virtual Presentation, September 2024.
- CFSRE's NPS Discovery Program. Alex J. Krotulski*. Global Coalition to Address Synthetic Drug Threats SWG 2.1, Virtual Presentation, May 2024.

- An Outlook on Fentanyl Adulteration: Polydrug Circumstances, Trends, and Tomorrow's Forecast. Alex J. Krotulski*, Sara E. Walton*, Donna M. Papsun, Joshua DeBord, Barry K. Logan American Academy of Forensic Sciences, Platform Presentation, February 2024.
- Confirming the Presence of Novel Psychoactive Substances in Forensic Samples from Medicolegal Death Investigations. Alex J. Krotulski*, Sara E. Walton*, Donna M. Papsun, Melissa F. Fogarty, Joshua DeBord, Barry K. Logan. NIJ Forensic Science R&D Symposium, American Academy of Forensic Sciences, Poster Presentation, February 2024.
- Novel Psychoactive Substances (NPS) – Landscape, Trends, and Current Polydrug Connections. Alex J. Krotulski*, ACMT Seminar in Forensic Toxicology, Expanding Worlds of Cannabinoids & Polydrug Exposures, Oral Presentation, December 2023.
- Implementation of CFSRE's NPS Discovery Program and Usefulness in Medicolegal Death Investigations. Alex J. Krotulski*, Forensic Toxicology Symposium, Centre for Forensic Science, Oral Presentation, November 2023.
- Comprehensive LC-QTOF-MS Analysis for NPS in a Complex Forensic Toxicology World, Alex Krotulski*, SCIEX Lunch & Learn Lightning Talks, Society of Forensic Toxicologists (SOFT), Platform Presentation, October 2023.
- CFSRE's NPS Discovery – A No-Cost Resource for Tracking and Confirming the Presence of Novel Psychoactive Substances in Forensic Samples from Medicolegal Death Investigations. Alex J. Krotulski*, Sara E. Walton, Donna M. Papsun, Melissa F. Fogarty, Joshua DeBord, Barry K. Logan, National Association of Medical Examiners, Oral Presentation, October 2023.
- The Role of North American Drug Early Warning System for Tracking and Combatting New Synthetic Opioids. Alex J. Krotulski*, Barry K. Logan, Second EMCDDA Technical Expert Meeting on New Synthetic Opioids, Oral Presentation, September 2023.
- Medicolegal Death Investigations Involving Novel Psychoactive Substances (NPS). Alex J. Krotulski*. Florida Association of Medical Examiners (FAME) Annual Education Conference, Oral Presentation, July 2023.
- The Current Landscape of Novel Psychoactive Substances (NPS) in the United States. Alex J. Krotulski*. Florida Association of Medical Examiners (FAME) Annual Education Conference, Oral Presentation, July 2023.
- Examining the Role of CFSRE's NPS Discovery Program. Alex J. Krotulski*. NMS Labs – Scientific Engagement Series, Virtual Presentation, July 2023.
- CFSRE's NPS Discovery – A Timely Drug Surveillance Program. Alex J. Krotulski*. NIJ Research Conference, Emerging Drug Crises in America: A Criminal Justice and Public Health Nexus, Presentation and Panel, May 2023.
- Impacts of Emerging Drugs and Novel Psychoactive Substances Tracked by CFSRE's NPS Discovery Open Access Drug Early Warning System. Alex Krotulski*. Medical Toxicology Grand Rounds, Midwest Toxicology Collaborative, Presentation, February 2023.
- An Unusual Case of Misrepresented Cocaine Powder That Resulted in Fatal and Non-Fatal Intoxications Involving the Novel Synthetic Opioid Etodesnitazene. Alex Krotulski*, Stephany Fiore, Daniel Anderson, Sara Walton, Brianna Peterson, Donna

Papsun, Joshua DeBord, Barry Logan. American Academy of Forensic Sciences, Platform Presentation, February 2023.

- Tracking and Disseminating Data on Novel Psychoactive Substances (NPS) through NPS Discovery's Drug Early Warning System. Alex J. Krotulski*, Sara E. Walton*, Donna M. Papsun, Melissa F. Fogarty, Barry K. Logan. NIJ Forensic Science R&D Symposium, American Academy of Forensic Sciences, Poster Presentation, February 2023.
- Updates from the CFSRE's NPS Discovery. Alex J Krotulski*. Current Trends in Seized Drug Analysis Symposium. Virtual Presentation, January 2023.

*2.2 Webinars and Workshops*

- Non-Targeted LC-QTOF-MS Analysis and Targeted LC-QQQ-MS Analysis of Novel Synthetic Opioids. Sara E. Walton*. Workshop 7, Society of Forensic Toxicologists, St. Louis, MO, 2024.
- Novel Psychoactive Substances – A Comprehensive Overview. Alex J. Krotulski* *(Plenary Speaker)*. Southeast Regional Overdose Prevention Coalition Conference 2024, Platform Presentation, October 2024.
- Analytical Characterization of Emerging Drugs – Practical Approaches to Address Forensic Science Challenges. Alex J. Krotulski*. JFS Forensic Lab Talks #7, Virtual Presentation, July 2024.
- Precursors, Byproducts, & Intermediates of NPS. Alex J. Krotulski*. The What, Where and How of Novel Psychoactive Substances, SOFT Conn Ed Webinar, Virtual Presentation, June 2024.
- Trending NPS in Casework. Alex J. Krotulski*. The What, Where and How of Novel Psychoactive Substances, SOFT Conn Ed Webinar, Virtual Presentation, June 2024.
- NPS Discovery Updates and Trend Reports for Emerging Drugs in 2024. Alex J. Krotulski*. The Role of Comprehensive Medicolegal Death Investigation as Part of a Public Health Improvement Strategy, CDC / NNPHI Webinar Series, Virtual Presentation, April 2024.
- Public Health's Response to the Overdose Crisis Using Front-Line Harm Reduction Initiatives to Gather Real-Time Intelligence. Alex J. Krotulski*, Workshop 19: Forensic Scientists on the Front Lines, American Academy of Forensic Sciences, Platform Presentation, February 2024.
- Use of Metabolite Profiling to Aid Forensic Toxicology Testing and Investigations. Alex J. Krotulski*, Improving NPS Forensic Testing with Metabolite Profiling, SCIEX and Wiley Webinar, Oral Presentation, November 2023.
- Developing an Interpretation Workflow for Toxicology Cases Involving NPS. Alex J. Krotulski*, Workshop 11: Forensic Interpretation of Novel Psychoactive Substances in Challenging Cases, Society of Forensic Toxicologists (SOFT), Platform Presentation, October 2023.
- Handling NPS Isomers – From Analytical Separation to Reporting. Melissa F. Fogarty, Sara E. Walton, Alex J. Krotulski*, Workshop 11: Forensic Interpretation of Novel Psychoactive Substances in Challenging Cases, Society of Forensic Toxicologists (SOFT), Platform Presentation, October 2023.

- Early NPS Case Involving New Synthetic Cannabinoids. Alex J. Krotulski*, Workshop 3: QTOF 101: A Guide to Successful Development and Validation, Society of Forensic Toxicologists (SOFT), Platform Presentation, October 2023.
- QTOF Data Acquisition – What to Know Before You Hit "Go". Alex J. Krotulski*, Workshop 11: Forensic Interpretation of Novel Psychoactive Substances in Challenging Cases, Society of Forensic Toxicologists (SOFT), Platform Presentation, October 2023.
- What's the Current State of the Synthetic Cannabinoid Market? Alex J. Krotulski*. Quarterly NPS Discovery Webinar Series, Virtual Presentation, October 2023.
- An Open Access Drug Early Warning System – Tracking Trends and Prevalence in the United States. Alex J. Krotulski*. National Association of Drug Diversion Investigators (NADDI) - Webinar Learning Series, Virtual Presentation, July 2023.
- Q2 2023 Updated Trend Reports and Positivity Plots. Alex J. Krotulski*. Quarterly NPS Discovery Webinar Series, Virtual Presentation, July 2023.
- Trends in the Emergence of Novel Psychoactive Substances. Barry K. Logan and Alex J. Krotulski*. Combined Surveillance Session – CDC "Overdose Data to Action" Recipient Meeting, Workshop Presentation, June 2023.

*2.3 General Press, Podcasts, and Other Media*

- Podcasts and Media – https://www.cfsre.org/nps-discovery/podcasts-and-media

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