Real-Time Sample-Mining and Data-Mining Approaches for the Discovery of Novel Psychoactive Substances (NPS)

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PROJECT SUMMARY (ORIGINAL AWARD)

1. Goals and Objectives

This research addressed the rapid identification of novel psychoactive substances (NPS) and resulted in the dissemination of information to combat emerging drug threats. During this award, we proposed to enhance utilization of novel approaches, termed "sample-mining" and "data-mining", for the analysis of emerging and previously unknown drugs in biological specimens acquired from various collaborators. Our developed methods have successfully identified 48 new substances and more than 100 continually detected substances, including opioids (e.g., fentanyls, U-series, benzimidazoles, opioid precursors), cannabinoids, stimulants (e.g., cathinones, amphetamines), hallucinogens, and benzodiazepines. This research was successful in expanding and improving workflows to rapidly acquire additional information pertinent to the study of NPS (e.g., metabolism, quantitation, drug combinations, poly-drug use).

1.1 Purpose, Aims, and Objective

The purpose of this research was to rapidly detect changes in NPS markets and to develop "toolkits" for use by practitioners detailing emerging NPS for which limited information is available, especially data regarding identity, analytical characteristics, prevalence, metabolism, and quantitative confirmation. For appropriate design and implementation, this research was divided into six key objectives:

1. **NPS Surveillance:** Develop a continuously updated testing panel using an expansive library database to include new substances 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 biological samples to detect and discover the newest substances present within forensically relevant populations, as well as to determine the most prevalent substances requiring further research and developments.
3. **NPS Metabolism:** Characterize metabolic pathways associated with new and prevalent NPS to identify the most appropriate biomarkers for different biological specimens to assist with future testing and to determine whether generated metabolites could contribute to effects or toxicity.
4. **NPS Confirmation:** Rapidly develop and validate 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 the above 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:** Immediately disseminate deliverables from this research to forensic science and criminal justice stakeholders via email and through our website.

Presentations at in person and on-line forensic science conferences are part of our dissemination strategy, as well as peer reviewed publication of key findings in appropriate forensic science journals.

The above model provided an important avenue for knowledge creation and transfer from our laboratory to other forensic laboratories performing forensic casework, including medicolegal death investigations and DUID investigations. This approach strengthened the forensic science community by relieving the burden on practicing laboratories to perform this research and development themselves. The NPS toolkits developed greatly decreased lag times for practicing laboratories to add these compounds to their scope(s) of analysis by providing detailed processes for streamlined development and analysis.

2. Research Questions

2.1 Research & Development Needs

The Organization of Scientific Area Committees (OSAC) for Forensic Science at the National Institute of Standards and Technology (NIST), has published research and development needs for forensic toxicology (1). This guidance describes the need for research involving emerging drugs of abuse (i.e., NPS) and therapeutic agents. Most specifically, the toxicology sub-committee encourages research involving the development of analytical methods for the identification and quantitation of NPS, metabolite identification assessments, and postmortem distribution studies. In addition, the committee endorses research that will allow for the development and optimization of sample preparation techniques that can be used by the forensic toxicology community. Research regarding NPS has been assessed as a major gap with limited research being conducted. These issues were expressly addressed in this research project.

The National Institute of Justice (NIJ) has investigated practitioners of various forensic disciplines and published research and development needs. NIJ priorities include increasing the body of knowledge to guide and inform forensic science policy and practice and to produce useful materials or methods that have potential for forensic application. This project aimed to address both objectives, providing tools and workflows designed to produce accurate, timely, and usable information about identification of emerging NPS that can guide and inform drug policy, including scheduling of drug substances and the refinement of federal, state, and local laws controlling NPS.

The deliverables produced and disseminated as part of this research have direct application in practicing forensic laboratories, including the generation of drug monographs, trend reports, public alerts, peer reviewed analytical methods, and workflows and toolkits to promote adoption in practicing forensic laboratories.

2.2 Knowledge Gaps

A primary knowledge gap for NPS relates to the true extent of NPS involvement in forensic investigations and the total number of new NPS substances on the market at a given

time. Current research and testing workflows do not allow for timely and accurate characterization of NPS in forensically relevant samples, leading to misidentification and under-reporting of these substances. To compound this issue, current approaches struggle to keep up to date with the new drug landscape. When a new NPS emerges, there is a need to rapidly describe the chemical signature, activity, potency, toxicity, and other general information about the substance to aid both toxicological and drug chemistry applications, as well as criminal justice and legal aspects associated. Additional knowledge gaps of significance to forensic toxicologists relate to the metabolism of a drug or NPS. To develop accurate toxicology testing panels, research on NPS metabolism is essential when a new drug begins to be popular, ensuring that the appropriate biomarker for its use can be identified and incorporated into drug testing methods.

2.3 Importance of Research

This research filled a gap in knowledge critical to forensic science practitioners to ensure early identification and detection of new substances contributing to illicit drug trafficking, deaths, and injuries in the United States. The United States lacks a comprehensive national early warning system for new drug identifications that accurately and quickly creates alerts about the emergence and spread of new synthetic drugs for forensic, clinical, and regulatory purposes. Forensic science and criminal justice stakeholders greatly benefit from the rapid discovery and dissemination of information to drug chemists, toxicologists, medical examiners, coroners, and law enforcement (2).

Many organizations need timely information on emerging substances to alert their stakeholders; so, while the infrastructure exists to share information, it is frequently not timely or comprehensive. For NPS, which generally have 6 to 9-month life cycles, very timely identification and reporting is essential. Programs that allow laboratories, law enforcement, and emergency medical services to rapidly identify and share data on emerging NPS associated with deaths and adverse events have been shown to be a key contributor to public safety and security. The European Union Drugs Agency (EUDA) is a drug monitoring organization founded to provide drug-related information to European public health agencies based on drug trends and drug use statistics (3). EUDA operates an early warning system to share information about NPS and through this system, EUDA has identified the emergence of more than 500 NPS (4). EUDA recognizes the use of illicit substances, including NPS, to be a “global burden of disease”.

2.4 Impacts

This project improved scientific knowledge and technical capabilities for laboratories through method development, validation, and implementation on various platforms and raised awareness of the threats from multiple NPS. The metabolite discovery aspects of the project helped laboratories make existing analytical approaches more targeted and relevant. This project involved the rapid development of new tests for NPS which directly assist in expanding technical capabilities among forensic laboratories who utilize the disseminated information.

Specific deliverables from the project included validated methods, structural information and drug characteristics, and metabolite identifications from *in vitro* experiments and *in vivo* testing. Electronic spectra of newly discovered compounds and their metabolites were

disseminated in electronic databases that can be utilized by practicing forensic laboratories. Disseminated trend reports continue to allow forensic scientists to determine the appropriate scope of testing when developing new panels or adding drugs to screening protocols. Reported drug concentration data, drug co-morbidities, and outcome data (e.g., cause and manner of death) assist forensic toxicologists, pathologists, and medical examiners, as well as physicians and medical toxicologists, in their work supporting public health and public safety.

3. Summary of Project Design and Methods

3.1 Objective 1: NPS Surveillance

The intent of this objective was to develop an up-to-date testing panel using an expansive library database to include new drugs available via standard reference material manufacturers to create a comprehensive scope of testing. The primary activity under this objective was the acquisition and analysis of standard reference materials by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Standard reference materials were purchased from qualified refence material vendors.

The addition of these NPS significantly expanded the library database and allowed for prospective monitoring of NPS in forensic casework samples to include new drug monographs and public alerts for NPS of concern. Appropriate additions to the library were determined based on intelligence streams (e.g., DEA and EUDA reports, communication with forensic colleagues, information from conference proceedings, information from scientific literature, online drug user forums, government scheduling actions, online illicit drug market activity, new pharmaceutical products, etc.).

Drug standards were analyzed by LC-QTOF-MS prior to library addition, which allowed for the verification of standards received, as well as generation of the in-house library database. Analysis was performed using a Sciex TripleTOF™ 5600+ and a X500R QTOF-MS coupled with a Shimadzu Nexera XR and Sciex Exion ultra-high performance liquid chromatograph, respectively. Gradient elution was performed using a standard reverse phase gradient and a Phenomenex® Kinetex C18 analytical column (50 x 3.0 mm, 2.6 µm) (5). Mobile phases were 10 mM ammonium formate in water, pH 3 (MPA) and 0.1% formic acid in methanol/acetonitrile, 50:50 *v:v* (MPB). The method described was utilized for all library additions, as well as authentic sample analysis.

The generation of the in-house library database included population of a mass list (or extracted ion chromatogram [XIC] list) and library file of fragment spectra. The XIC list included data such as retention time, formula, exact mass, accurate fragment masses (n=5), and general software required settings. The information compiled from this analysis was consolidated into an Excel spreadsheet and into the LibraryView software.

3.2 Objective 2: NPS Monitoring

A critical aspect of this research was the testing of authentic forensic samples for identification of new substances, and determination of their positivity and prevalence. This research is largely lacking and as mentioned above, is a priority area of research identified by the OSAC Toxicology Subcommittee and the NIJ. The only way to determine the true extent of NPS prevalence and impact is by analysis of representative populations, particularly those that may be rich sources of information regarding substance use and emerging trends. The main activity for this objective was to use prospective and retrospective approaches for NPS discovery by testing authentic biological samples in forensically relevant populations.

To conduct sample-mining, our laboratory partnered with other laboratories and agencies to acquire biological specimens and sample extracts from forensic casework. Biological samples typically included blood and urine samples, but other matrices were accepted if available. Upon receipt, biological samples were prepared using basic and acidic liquid-liquid extractions to encompass the large variety of NPS that are involved in poly-drug overdoses. Basic liquid-liquid extraction was performed using borax buffer (1 mL, 10 mM, pH 10.4) and an extraction solvent of *N*-butyl chloride/ethyl acetate (3 mL, 70:30, *v:v*). Acidic liquid-liquid extraction 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*) (6, 7). Following extraction, samples were analyzed using LC-QTOF-MS as described above. Sample extracts were analyzed "as received." The results for all drugs identified were compiled into an Excel spreadsheet and used for dissemination by reports on the website or peer-reviewed publications.

3.3 Objective 3: NPS Metabolism

To map the metabolism of emerging NPS, a battery of experiments and testing workflows were used for comprehensive and accurate characterization. *In vitro* assessment of metabolism was studied using pooled human liver microsomes (HLMs). This approach produces an array of metabolic species (~10-30) which can be representative of what will be identified within human biological specimens; however, further confirmation of HLM generated metabolites is encouraged.

Metabolism studies were performed for drugs of interest alongside a control (diazepam) over three days. 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 (6). Incubations were performed at 37°C and analysis was performed via LC-QTOF-MS as previously described. Data were processed using MetabolitePilot™ and consolidated into an Excel spreadsheet.

After metabolism studies were completed, authentic human biological specimens (e.g. urine, blood) were analyzed from cases where the ingestion of the parent NPS is confirmed, if available, using extraction and analysis protocols previously described. This allows for corroborative identification of the metabolites and correlation between *in vitro* experiments and *in vivo* testing. Final characterization included testing a population of biological specimens to determine overall prevalence of the NPS metabolites (if available), which allowed us to

distinguish major and minor metabolites. This final process is critical for informing forensic scientists about recommendations for NPS metabolites to add to drug testing scopes.

3.4 Objective 4: NPS Confirmation

Confirmation methods for the most prevalent NPS identified through surveillance and monitoring were developed using multi-preparation and multi-platform approaches, when possible, for better coverage of methodologies and instrumentation commonly encountered in forensic laboratories. Six confirmation methods for NPS were developed, validated, and published during the project period. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) sample preparation protocols were developed and evaluated to determine feasibility with emerging NPS. Liquid chromatography tandem mass spectrometry (LC-QQQ-MS) is the most used instrument for NPS confirmation; however, multiple instrument platforms were used to determine optimum detection parameters. In addition, gas chromatography mass spectrometry (GC-MS) was evaluated, if applicable. This research, and its subsequent dissemination, reduces the burden on forensic laboratories, expediting the implementation of methods.

The development of quantitative confirmation methods allowed our laboratory to develop reference concentration data for emerging NPS in forensic toxicology samples. 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. The primary matrix acquired in these partnerships was blood; however, urine, serum/plasma, and other alternative matrices were accepted if available.

Methodologies for the confirmation and quantitation of metonitazene, 5F-MDMB-PICA and metabolites, nitazene analogues (e.g., isotonitazene, protonitazene, etc.), cinnamylpiperazines (e.g., AP-238, 2-methyl AP-237), *N*-pyrrolidino etonitazene, and *N*-piperidinyl etonitazene were developed (5, 6, 8–11). Methods were validated according to the AAFS Standards Board (ASB) Standard 036 (12). NPS testing panels were developed based on newly emerging NPS and were adapted as necessary. Sample preparation was performed via basic or acidic LLE and instrumental analysis was performed using LC-QQQ-MS. 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 fifty-three drug standards were added to the scope of analysis during the research period of 2021 to 2022 (**Table 1**). This database expansion exceeded the original goal of the addition of 50-100 new NPS based on the historical emergence of NPS and were primarily purchased from Cayman Chemical Company. Using the expanded library database for the surveillance of new NPS, 48 new drug monographs (**Table 2**) were issued. In addition to the deliverables proposed in the solicitation, three NPS scope recommendations were added to

include surveillance of NPS across the United States in collaboration with the Society of Forensic Toxicologists' NPS Committee (https://www.cfsre.org/nps-discovery/scope-recommendations).

Table 1: New drug standards added to library database.

#	Drug Name	Chemical Formula	[M+H]+ (Da)
1	4-(Trifluoromethyl) U-47700	$C_{17}H_{23}F_3N_2O$	329.1835
2	Nitrazolam	$C_{17}H_{13}N_5O_2$	320.1142
3	Flunitrazolam	$C_{17}H_{12}FN_5O_2$	338.1048
4	Clonazolam	$C_{17}H_{12}ClN_5O_2$	354.0752
5	Meclonazepam	$C_{16}H_{12}ClN_3O_3$	330.064
6	*N*-desethyl Etonitazene	$C_{20}H_{24}N_4O_3$	369.1921
7	*ortho*-Fluorofentanyl	$C_{22}H_{27}FN_2O$	355.218
8	*meta*-Fluorofentanyl	$C_{22}H_{27}FN_2O$	355.218
9	*ortho*-chlorofentanyl	$C_{22}H_{27}ClN_2O$	371.1885
10	*meta*-Chlorofentanyl	$C_{22}H_{27}ClN_2O$	371.1885
11	*para*-Chlorofentanyl	$C_{22}H_{27}ClN_2O$	371.1885
12	3-fluoro-*N*-ethyl Hexedrone	$C_{14}H_{20}FNO$	238.1602
13	Tenocyclidine	$C_{15}H_{23}NS$	250.1624
14	Suvorexant	$C_{23}H_{23}ClN_6O_2$	451.1644
15	DMPEA	$C_{10}H_{15}NO_2$	182.1176
16	4OH-ADB-BINACA	$C_{18}H_{26}N_4O_3$	347.2078
17	4F-MDMB-BICA 3,3-Dimethylbutanoic Acid	$C_{19}H_{25}FN_2O_3$	349.1922
18	ADB-BINACA *N*-butanoic Acid	$C_{18}H_{24}N_4O_4$	361.187
19	2Br-DMPEA	$C_{10}H_{14}BrNO_2$	260.0281
20	4OH-MDMB-BICA	$C_{20}H_{28}N_2O_4$	361.2122
21	4F-ABUTINACA *N*-butanoic Acid	$C_{22}H_{27}N_3O_3$	382.2125
22	*N*-Pyrrolidino Etonitazene	$C_{22}H_{26}N_4O_3$	395.2078
23	ADB-4en-PINACA	$C_{19}H_{26}N_4O_2$	343.2129
24	4CN-MMB-BUTINACA	$C_{19}H_{24}N_4O_3$	357.1921
25	5-MeO MiPT	$C_{15}H_{22}N_2O$	247.1805
26	25I-NBOH	$C_{17}H_{20}INO_3$	414.0561
27	3,4-Methylenedioxy PV8	$C_{18}H_{25}NO_3$	304.1907
28	Desalkylflurazepam	$C_{15}H_{10}ClFN_2O$	289.0538
29	MDDMA	$C_{12}H_{17}NO_2$	208.1332
30	Delta-8-THC	$C_{21}H_{28}O_4$	345.206
31	Tiletamine	$C_{12}H_{17}NOS$	224.1104
32	*alpha*-PipBP	$C_{15}H_{21}NO$	232.1696
33	*para*-fluoro Phenethyl 4-ANPP	$C_{27}H_{31}FN_2$	403.2544
34	Brotizolam	$C_{15}H_{10}BrClN_4S$	392.9571
35	ABO-4en-PINACA	$C_{17}H_{22}N_4O_2$	315.1816
36	O-AMKD	$C_{16}H_{21}NO_3$	276.1594
37	2-Naphthyl U-47700	$C_{20}H_{26}N_2O$	311.2118
38	*N*-Piperidinyl Etonitazene	$C_{23}H_{28}N_4O_3$	409.2234
39	Bipiperidinyl 4-ANPP	$C_{24}H_{33}N_3$	364.2747
40	EDMB-PINACA	$C_{21}H_{31}N_3O_3$	374.2438
41	*N*-methyl-2-AI	$C_{10}H_{13}N$	148.1121
42	*alpha*-D2PV	$C_{18}H_{19}NO$	266.1539
43	ADB-PHETINACA	$C_{22}H_{26}N_4O_2$	379.2129
44	*N*-propionyl Norfentanyl	$C_{17}H_{24}N_2O_2$	289.1911
45	MMB-FUBGACLONE	$C_{24}H_{23}FN_2O_3$	407.1765
46	Flubrotizolam	$C_{15}H_{10}BrFN_4S$	376.9866
47	Dipyanone	$C_{23}H_{29}NO$	336.2322
48	Hydroxetamine	$C_{14}H_{19}NO_2$	234.1489
49	4-acetoxy EPT	$C_{17}H_{24}N_2O_2$	289.1911

50	5F-BZO-POXIZID	$C_{20}H_{20}FN_3O_2$	354.1612
51	BZO-POXIZID	$C_{20}H_{21}N_3O_2$	336.1707
52	3-fluoro-*N*-Ethylbuphedrone	$C_{12}H_{16}FNO$	210.1289
53	3-methyl PCP	$C_{18}H_{27}N$	258.2216
54	3-methoxy PCPy	$C_{17}H_{25}NO$	260.2009
55	Deoxymethoxetamine	$C_{15}H_{21}NO$	232.1696
56	3-Chlorocathinone	$C_9H_{10}ClNO$	184.0524
57	CUMYL-NBMICA	$C_{26}H_{30}N_2O$	387.2431
58	3-Chloromethcathinone	$C_{10}H_{12}ClNO$	198.0680
59	*N*-butyl Pentylone	$C_{16}H_{23}NO_3$	278.3493
60	3,4-Dichloroethcathinone	$C_{11}H_{13}Cl_2NO$	247.1073
61	4-Fluoroethamphetamine	$C_{11}H_{16}FN$	182.2073
62	4-Cl-*N,N*-Dimethylcathinone	$C_{11}H_{14}ClNO$	213.0073
63	Flubromazolam	$C_{17}H_{12}BrFN_4$	372.2073
64	*para*-Bromofentanyl	$C_{22}H_{27}BrN2O$	416.4073
65	ADB-FUBIATA	$C_{23}H_{26}FN_3O_2$	396.2082
66	4CN-AB-BICA	$C_{19}H_{24}N_4O_2$	341.1972
67	BZO-CHMOXIZID	$C_{22}H_{23}N_3O_2$	362.1863
68	2-Br-Deschloroketamine	$C_{13}H_{16}BrNO$	282.0488
69	MDMB-BENZICA	$C_{23}H_{26}N_2O_3$	379.2016
70	Fluorexetamine	$C_{14}H_{18}FNO$	236.1445
71	Dermorphin	$C_{40}H_{50}N_8O_{10}$	803.3723
72	Meptazinol*	$C_{15}H_{23}NO$	234.1852
73	ADB-FUBIATA 3,3-dimethylbutanoic acid	$C_{23}H_{25}FN_2O_3$	397.1922
74	*N*-propyl butylone	$C_{14}H_{19}NO_3$	250.1438
75	2-Methylmethcathinone	$C_{11}H_{15}NO$	178.1226
76	3-Methylmethcathinone	$C_{11}H_{15}NO$	178.1226
77	Mephedrone	$C_{11}H_{15}NO$	178.1226
78	ADB-5Br-INACA	$C_{14}H_{17}BrN_4O_2$	353.0608
79	4'-Cl-Deschloroalprazolam	$C_{17}H_{13}ClN_4$	309.0902
80	BZO-4en-POXIZID	$C_{20}H_{19}N_3O_2$	334.1550
81	3,5-ADB-4en-PFUPPYCA	$C_{21}H_{27}FN_4O_2$	387.2191
82	*N*-cyclohexyl-*N*-methyl Methylone	$C_{17}H_{23}NO_3$	290.1751
83	Chlorphine	$C_{20}H_{22}ClN_3O$	356.1524
84	Orphine	$C_{20}H_{23}N_3O$	322.1914
85	Fluorphine	$C_{20}H_{22}FN_3O$	340.1820
86	MDMB-5Br-INACA	$C_{15}H_{18}BrN_3O_3$	368.0604
87	Nitazene	$C_{20}H_{24}N_4O_2$	353.1972
88	Menitazene	$C_{21}H_{26}N_4O_2$	351.2179
89	Acepromazine	$C_{19}H_{22}N_2OS$	327.1526
90	Fluetizolam	$C_{17}H_{15}FN_4S$	327.1074
91	*N*-Cyclohexyl Methylone	$C_{16}H_{21}NO_3$	276.1594
92	CH-PIATA	$C_{21}H_{30}N_2O$	327.2431
93	*N*-ethyl-*N*-methyl Butylone	$C_{14}H_{19}NO_3$	250.1438
94	*N*-isopropyl Butylone	$C_{14}H_{19}NO_3$	250.1438
95	*alpha*-hydroxy Clonazolam	$C_{17}H_{12}ClN_5O_3$	370.0701
96	ADB-BUTINAATA	$C_{19}H_{28}N_4O_2$	345.2285
97	ADB-5'Br-BUTINACA	$C_{18}H_{25}BrN_4O_2$	409.1234
98	5-methyl Etodesnitazene	$C_{23}H_{31}N_3O$	366.2540
99	*N*-cyclohexyl Butylone	$C_{17}H_{23}NO_3$	290.1750
100	CH-FUBIATA	$C_{23}H_{25}FN_2O$	365.2024
101	Menitazene	$C_{21}H_{26}N_4O2$	351.2179
102	MMB-5Br-INACA	$C_{14}H_{16}BrN_3O_3$	354.0448
103	CUMYL-TsINACA	$C_{24}H_{23}N_3O_3S$	434.1533
104	AFUBIATA	$C_{27}H_{29}FN_2O$	417.2337
105	MDMB-INACA	$C_{15}H_{19}N_3O_3$	290.1499
106	Dipipanone	$C_{24}H_{31}NO$	350.2478
107	Desalkylgidazepam	$C_{15}H_{11}BrN_2O$	315.0128
108	2-fluoro-2-oxo PCE	$C_{14}H_{18}FNO$	236.1445

109	Ethyl 4-ANPP	$C_{21}H_{28}N_2$	309.2325
110	Protodesnitazene	$C_{23}H_{31}N_3O$	366.2540
111	3,4-Methylenedioxy PiHP	$C_{17}H_{23}NO_3$	290.1751
112	*N*-Pyrrolidino Isotonitazene	$C_{23}H_{28}N_4O_3$	409.2234
113	*N*-Piperidinyl Isotonitazene	$C_{24}H_{30}N_4O_3$	423.2391
114	5-Trifluoromethyl Isotonitazene	$C_{24}H_{30}F_3N_3O$	434.2414
115	*sec*-Butonitazene	$C_{24}H_{32}N_4O_3$	425.2547
116	*iso*-Butonitazene	$C_{24}H_{32}N_4O_3$	425.2547
117	*alpha*-Methyl Etonitazene	$C_{23}H_{30}N_4O_3$	411.2391
118	Methionitazene	$C_{21}H_{26}N_4O_2S$	399.1849
119	Ethylene Etonitazene	$C_{23}H_{30}N_4O_3$	411.2391
120	Propylnitazene	$C_{23}H_{30}N_4O_2$	395.2442
121	Etoetonitazene	$C_{24}H_{32}N_4O_4$	441.2496
122	Pyrrolidino Variant Etonitazene	$C_{23}H_{28}N_4O_3$	409.2234
123	Nitazene Dihydrofuran Variant	$C_{22}H_{26}N_4O_3$	395.2078
124	3,4,5-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
125	*N*-Pyrrolidino Protonitazene	$C_{23}H_{28}N_4O_3$	409.2234
126	3,4-methylenedioxy PCP	$C_{18}H_{25}NO_2$	288.1958
127	5-MAPB	$C_{12}H_{15}NO$	190.1226
128	*N*-Pyrrolidino Metonitazene	$C_{21}H_{24}N_4O_3$	381.1921
129	CUMYL-INACA	$C_{17}H_{17}N_3O$	280.1444
130	ADB-5'Br-PINACA	$C_{19}H_{27}BrN_4O_2$	423.1390
131	Tryptamine	$C_{10}H_{12}N_2$	161.1073
132	1,2,3,4-Tetrahydro-beta-carboline	$C_{11}H_{12}N_2$	173.1073
133	2,3,4-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
134	2-Methyl-1,2,3,4-Tetrahydro-beta-carboline	$C_{12}H_{14}N_2$	187.1230
135	2,3,5-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
136	3-Methylnordiazepam	$C_{16}H_{13}ClN_2O$	285.0789
137	*N,N*-DMT N-Oxide	$C_{12}H_{16}N_2O$	205.1336
138	Kavain	$C_{14}H_{14}O_3$	231.1016
139	2,3,6-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
140	Delta-9-THCO	$C_{17}H_{22}O_2$	259.1693
141	Delta-9-THCE	$C_{18}H_{24}O_2$	273.1849
142	2,4,5-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
143	ADB-INACA	$C_{14}H_{18}N_4O_2$	275.1503
144	Delta-9-THCB	$C_{20}H_{28}O_2$	301.2162
145	Delta-9-THCH	$C_{22}H_{32}O_2$	329.2475
146	THCVA-A	$C_{20}H_{26}O_4$	331.1904
147	THCA-A	$C_{22}H_{30}O_4$	359.2217
148	ADB-5'Br-4en-PINACA	$C_{19}H_{25}BrN_4O_2$	421.1234
149	2,4,6-Trimethoxyamphetamine	$C_{12}H_{19}NO_3$	226.1438
150	Delta-4(8)-iso-THC	$C_{21}H_{30}O_2$	315.2319
151	Delta-9-THC Methyl Ether	$C_{22}H_{32}O_2$	329.2475
152	Delta-9-THC-C8	$C_{24}H_{36}O_2$	357.2788
153	Indole-3-acetic Acid	$C_{10}H_9NO_2$	175.0628

**Purchased from Sigma Aldrich. All others purchased from Cayman Chemical.*

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

#	Name	NPS Class	Sample Type	Date Released
1	Butonitazene	Opioid	Blood	01/15/2021
2	4F-ABINACA	Cannabinoid	Plant-like material	01/27/2021
3	Cl-PCP	Hallucinogen	Blood	02/04/2021
4	F-PCP	Hallucinogen	Blood	02/04/2021
5	Deschloroetizolam	Benzodiazepine	Rectangular yellow tablet	02/16/2021
6	Etodesnitazene	Opioid	Blood	02/23/2021

7	ADB-4en-PINACA	Cannabinoid	Plant-like material	03/04/2021
8	Flunitazene	Opioid	Blood	03/26/2021
9	Methylenedioxy-PV8	Stimulant	Blood	04/23/2021
10	ADB-HEXINACA	Cannabinoid	Plant-like material	04/29/2021
11	*N*-Pyrrolidino Etonitazene	Opioid	Blood	05/13/2021
12	Protonitazene	Opioid	Blood	05/26/2021
13	4-AcO-EPT	Hallucinogen	Tan solid material	06/29/2021
14	Metodesnitazene	Opioid	Blood	09/22/2021
15	BZO-HEXOXIZID	Cannabinoid	Plant-like material	10/19/2021
16	BZO-POXIZID	Cannabinoid	Plant-like material	10/19/2021
17	5F-BZO-POXIZID	Cannabinoid	Plant-like material	10/19/2021
18	Fluclotizolam	Benzodiazepine	Urine	11/05/2021
19	ADB-FUBIATA	Cannabinoid	Plant-like material	11/17/2021
20	BZO-CHMOXIZID	Cannabinoid	Plant-like material	11/18/2021
21	ADB-PHETINACA	Cannabinoid	Serum	11/19/2021
22	*N*-Piperidinyl Etonitazene	Opioid	Serum	11/22/2021
23	Trifluoromethyl-U-47700	Opioid	Blood	12/14/2021
24	Tenocyclidine	Hallucinogen	Serum	12/15/2021
25	Napthyl-U-47700	Opioid	White solid material	12/16/2021
26	*N,N*-Dimethylpentylone	Stimulant	Blood	12/17/2021
27	EDMB-PINACA	Cannabinoid	Paper	12/20/2021
28	Dipyanone	Opioid	White solid material	03/14/2022
29	CH-PIATA	Cannabinoid	Plant-like material	04/29/2022
30	*N*-Cyclohexyl Methylone	Stimulant	Off-white crystalline powder	05/09/2022
31	ADB-5Br-INACA	Cannabinoid	Plant-like material	05/17/2022
32	MDMB-5Br-INACA	Cannabinoid	Plant-like material	05/17/2022
33	BZO-4en-POXIZID	Cannabinoid	Plant-like material	05/19/2022
34	ADB-5'Br-INACA	Cannabinoid	Plant-like material	05/26/2022
35	*N*-Cyclohexyl Butylone	Stimulant	White crystalline powder	06/09/2022
36	CH-FUBIATA	Cannabinoid	Plant-like material	06/21/2022
37	*N*-Propyl Butylone	Stimulant	White powder	07/21/2022
38	Hydroxetamine (HXE)	Hallucinogen	Blood	08/31/2022
39	Phenazolam	Benzodiazepine	Blood	12/06/2022
40	4'Cl-Deschloroalprazolam	Benzodiazepine	Blood	12/07/2022
41	Fluetizolam	Benzodiazepine	Plasma	12/08/2022
42	Desalkylgidazepam	Benzodiazepine	Blood	12/09/2022
43	CUMYL-TsINACA	Cannabinoid	Plasma	12/13/2022
44	ADB-INACA	Cannabinoid	White solid material	12/14/2022
45	Methylmethcathinone (MMC)	Stimulant	Blood	12/15/2022
46	Fluorexetamine (FXE)	Hallucinogen	Blood	12/16/2022
47	*N*-Desethyl Isotonitazene	Opioid	Round blue pill	12/19/2022
48	Trimethoxyamphetamine	Stimulant	Round green tablet	12/30/2022

4.2 Objective 2: NPS Monitoring

During the project period, our laboratory received 219 forensic toxicology specimens from medicolegal death investigations (MDI), driving under the influence of drugs (DUID) investigations, clinical intoxication investigations, and mass outbreak or overdose investigations, as well as other areas or circumstances that arose during our collaborations. In partnership with NMS Labs, our laboratory also received and tested 6,625 sample extracts for the presence of NPS during the project period. From these sample populations, 101 different NPS were identified in 2021 with 3,316 total identifications and 76 different NPS were identified in 2022 with 2,231 total identifications (**Table 3**). Using the data acquired from NPS monitoring, 16

trend reports depicting NPS positivity (**Figures 1-4**), eight public alerts (**Table 4**), and two Years in Review reports (**Figures 5-6**) were issued.

Table 3: Total NPS identifications in 2021 and 2022.

2021			2022		
Drug Name	**NPS Subclass**	**#**	**Drug Name**	**NPS Subclass**	**#**
Adinazolam	Benzodiazepine	12	4'Cl-Deschloroalprazolam	Benzodiazepine	22
Bromazepam	Benzodiazepine	15	Bromazepam	Benzodiazepine	12
Bromazolam	Benzodiazepine	91	Bromazolam	Benzodiazepine	156
Clonazolam	Benzodiazepine	357	Clonazolam	Benzodiazepine	70
Delorazepam	Benzodiazepine	13	Desalkylflurazepam	Benzodiazepine	14
Desalkylflurazepam	Benzodiazepine	26	Desalkylgidazepam	Benzodiazepine	7
Deschloroetizolam	Benzodiazepine	30	Deschloroetizolam	Benzodiazepine	37
Diclazepam	Benzodiazepine	4	Diclazepam	Benzodiazepine	1
Estazolam	Benzodiazepine	2	Estazolam	Benzodiazepine	1
Etizolam	Benzodiazepine	1010	Etizolam	Benzodiazepine	461
Flualprazolam	Benzodiazepine	429	Flualprazolam	Benzodiazepine	380
Flubromazepam	Benzodiazepine	50	Flubromazepam	Benzodiazepine	146
Flubromazolam	Benzodiazepine	170	Flubromazolam	Benzodiazepine	64
Fluclotizolam	Benzodiazepine	5	Fluclotizolam	Benzodiazepine	1
Meclonazepam	Benzodiazepine	9	Fluetizolam	Benzodiazepine	1
Metizolam	Benzodiazepine	8	Meclonazepam	Benzodiazepine	6
Phenazepam	Benzodiazepine	3	Phenazepam	Benzodiazepine	2
Pyrazolam	Benzodiazepine	5	Phenazolam	Benzodiazepine	1
4F-ABINACA	Cannabinoid	7	Pyrazolam	Benzodiazepine	6
4F-MDMB-BICA	Cannabinoid	21	4-cyano CUMYL-BUTINACA	Cannabinoid	4
4F-MDMB-BINACA	Cannabinoid	8	4F-MDMB-BINACA	Cannabinoid	3
5F-ADB	Cannabinoid	6	5F-ADB	Cannabinoid	3
5F-BZO-POXIZID	Cannabinoid	1	5F-MDMB-PICA	Cannabinoid	6
5F-EDMB-PICA	Cannabinoid	2	ADB-4en-PINACA	Cannabinoid	1
5F-MDMB-PICA	Cannabinoid	52	ADB-5'Br-BINACA	Cannabinoid	1
ACHMINACA	Cannabinoid	2	ADB-5Br-INACA	Cannabinoid	1
ADB-4en-PINACA	Cannabinoid	5	ADB-BINACA	Cannabinoid	17
ADB-BINACA	Cannabinoid	32	ADB-FUBIATA	Cannabinoid	1
ADB-FUBIATA	Cannabinoid	1	ADB-INACA	Cannabinoid	2
ADB-HEXINACA	Cannabinoid	1	BZO-4en-POXIZID	Cannabinoid	1
ADB-PHETINACA	Cannabinoid	1	CH-FUBIATA	Cannabinoid	1
BZO-CHMOXIZID	Cannabinoid	1	CH-PIATA	Cannabinoid	2
BZO-HEXOXIZID	Cannabinoid	1	CUMYL-TsINACA	Cannabinoid	1
BZO-POXIZID	Cannabinoid	1	MDMB-4en-PINACA	Cannabinoid	44
EDMB-PINACA	Cannabinoid	1	MDMB-5Br-INACA	Cannabinoid	1
MDMB-4en-PINACA	Cannabinoid	89	MMB-FUBINACA	Cannabinoid	3
2F-Deschloroketamine	Hallucinogen	5	3-HO-PCE	Hallucinogen	4
3-HO-PCE	Hallucinogen	1	3-OH-PCP	Hallucinogen	4
3-OH-PCP	Hallucinogen	4	4-HO-DiPT	Hallucinogen	1
4-AcO-EPT	Hallucinogen	1	4-MeO-PCP	Hallucinogen	2
4-HO-DiPT	Hallucinogen	2	Cl-PCP	Hallucinogen	2
4-MeO-PCP	Hallucinogen	2	Deschloroketamine	Hallucinogen	3
Cl-PCP	Hallucinogen	6	Fluorexetamine (FXE)	Hallucinogen	3
Deschloroketamine	Hallucinogen	3	Hydroxetamine (HXE)	Hallucinogen	1
F-PCP	Hallucinogen	1	2-methyl AP-237	Opioid	4
N-ethyl Deschloroketamine	Hallucinogen	1	Acetyl Fentanyl	Opioid	37
Tenocyclidine	Hallucinogen	1	Bromofentanyl	Opioid	5
Furanyl UF-17	Miscellaneous	7	Brorphine	Opioid	10
UF-17	Miscellaneous	5	Butryl Fentanyl	Opioid	1

2',5'-Dimethoxyfentanyl	Opioid	2
2-methyl AP-237	Opioid	17
2-Naphthyl U-47700	Opioid	1
4-(Trifluoromethyl) U-47700	Opioid	6
Acetyl Fentanyl	Opioid	114
AP-238	Opioid	2
Bromofentanyl	Opioid	1
Brorphine	Opioid	7
Butonitazene	Opioid	1
Carfentanil	Opioid	14
Chlorofentanyl	Opioid	5
Etodesnitazene	Opioid	7
Flunitazene	Opioid	1
Fluorofentanyl	Opioid	286
Isotonitazene	Opioid	8
Methoxyacetyl Fentanyl	Opioid	1
Metodesnitazene	Opioid	1
Metonitazene	Opioid	25
Napthyl-U-47700	Opioid	1
N-Piperidinyl Etonitazene	Opioid	1
N-Pyrrolidino Etonitazene	Opioid	20
para-Methyl Tetrahydrofuranylfentanyl	Opioid	6
Protonitazene	Opioid	10
Tianeptine	Opioid	12
Trifluoromethyl-U-47700	Opioid	1
U-47700	Opioid	1
U-51754/U-48800	Opioid	1
ValerylFentanyl	Opioid	1
3-Chlorocathinone	Stimulant	1
4Cl-*alpha*-PVP	Stimulant	1
alpha-D2PV	Stimulant	2
Alpha-PBP	Stimulant	1
Alpha-PHP/*Alpha*-PiHP	Stimulant	9
Alpha-PPP	Stimulant	1
Alpha-PVP	Stimulant	1
Benzylone	Stimulant	1
Butylone	Stimulant	1
Dibutylone	Stimulant	1
Dimethylone	Stimulant	2
Ethylphenidate	Stimulant	2
Eutylone	Stimulant	196
Fluoromethamphetamine	Stimulant	2
Methcathinone	Stimulant	2
Methylenedioxy-*alpha*-PHP	Stimulant	2
Methylenedioxy-PV8	Stimulant	1
Methylone	Stimulant	1
N,N-Dimethylpentylone	Stimulant	11
N-benzyl-3,4-DMA	Stimulant	1
N-butyl Pentylone	Stimulant	1
N-Ethyl Hexedrone	Stimulant	2
N-ethyl Pentedrone	Stimulant	3
Pentylone	Stimulant	8

Carfentanil	Opioid	28
Dipyanone	Opioid	1
Etodesnitazene	Opioid	4
Fluorofentanyl	Opioid	373
Isotonitazene	Opioid	18
Metonitazene	Opioid	22
N-Desethyl Isotonitazene	Opioid	6
N-Pyrrolidino Etonitazene	Opioid	7
Protonitazene	Opioid	6
Tianeptine	Opioid	5
alpha-D2PV	Stimulant	1
Alpha-PHP / *Alpha*-PiHP	Stimulant	10
Alpha-PVP	Stimulant	2
Benzylone	Stimulant	1
Butylone	Stimulant	1
Dimethylone	Stimulant	1
Eutylone	Stimulant	22
MDPV	Stimulant	2
Methedrone	Stimulant	1
Methylmethcathinone	Stimulant	1
N,N-Dimethylpentylone	Stimulant	94
N-Cyclohexyl Butylone	Stimulant	1
N-Cyclohexyl Methylone	Stimulant	1
N-Ethyl Pentylone	Stimulant	2
N-Propyl Butylone	Stimulant	3
Pentylone	Stimulant	60
Trimethoxyamphetamine	Stimulant	1

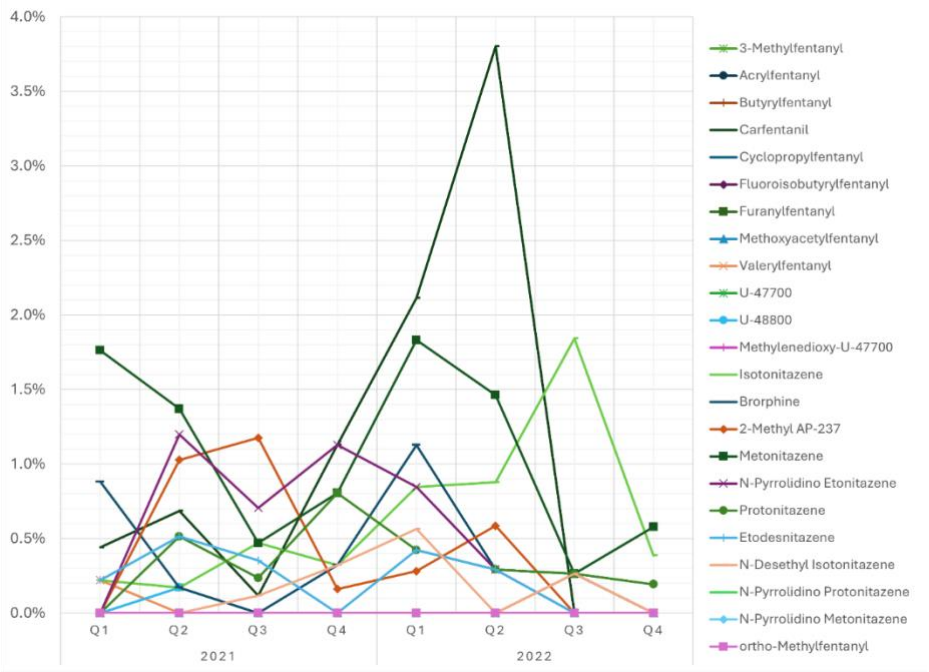

Figure 1: Positivity plot showing NPS opioids in toxicology samples analyzed by LC-QTOF-MS between Q1 2021 and Q4 2022 (https://www.cfsre.org/nps-discovery/trend-reports).

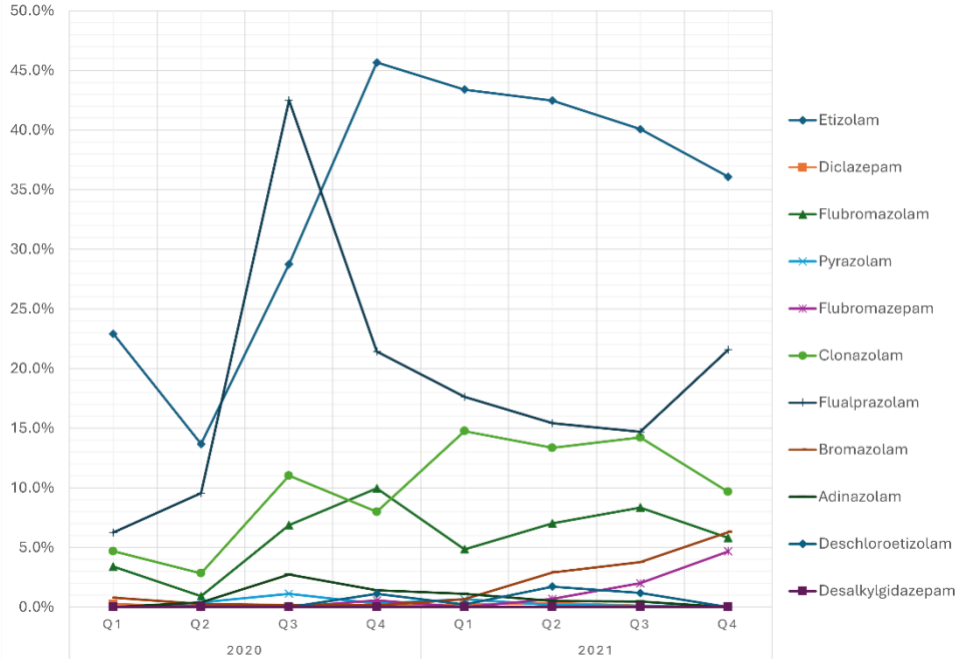

Figure 2: Positivity plot showing NPS benzodiazepines in toxicology samples analyzed by LC-QTOF-MS between Q1 2021 and Q4 2022 (https://www.cfsre.org/nps-discovery/trend-reports).

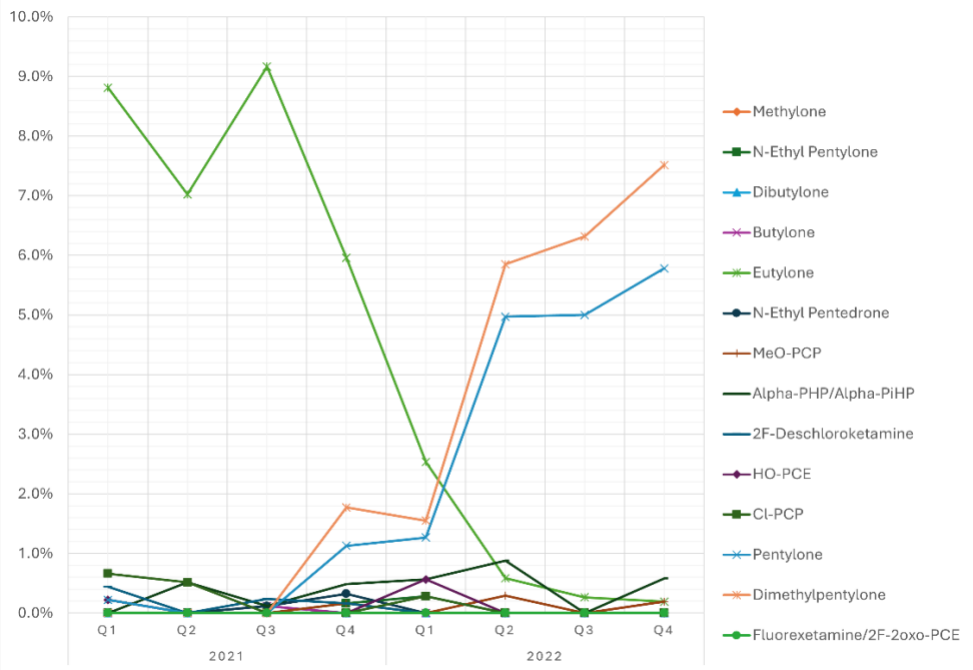

Figure 3: Positivity plot showing NPS stimulants and hallucinogens in toxicology samples analyzed by LC-QTOF-MS between Q1 2021 and Q4 2022 (https://www.cfsre.org/nps-discovery/trend-reports).

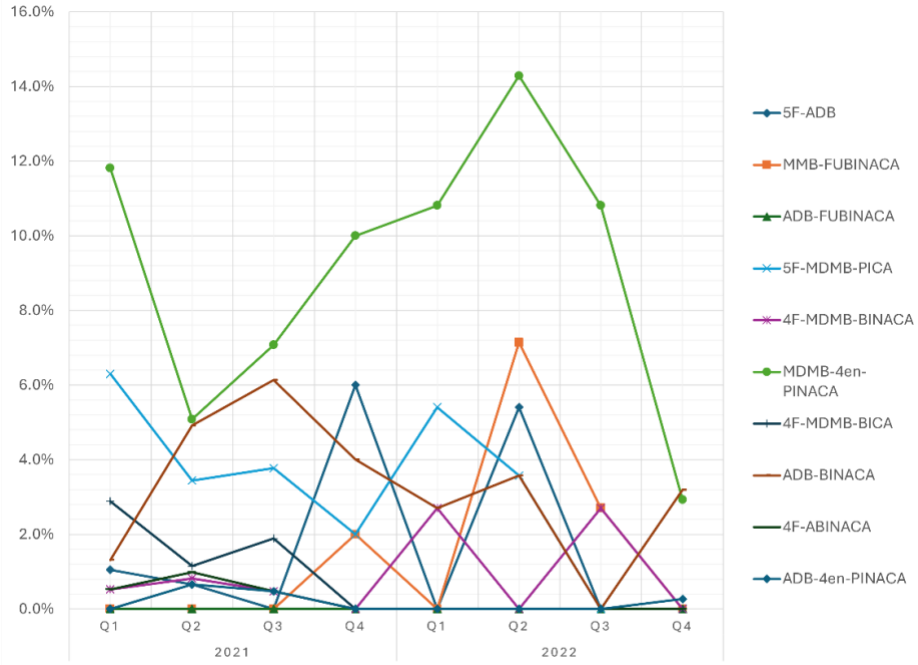

Figure 4: Positivity plot showing synthetic cannabinoids in toxicology samples analyzed by LC-QTOF-MS between Q1 2021 and Q4 2022 (https://www.cfsre.org/nps-discovery/trend-reports).

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

#	Name	Date Released	Reason for Alert
1	Metonitazene	01/25/2021	8 fatal overdoses
2	4F-MDMB-BICA	02/08/2021	22 fatal overdoses and 1 non-fatal overdose
3	*N*-Pyrrolidino Etonitazene	06/17/2021	8 fatal overdoses
4	New Generation Synthetic Cannabinoids (OXIZIDs)	08/31/2021	Class-wide legislation for synthetic cannabinoids
5	Protonitazene	12/10/2021	9 fatal overdoses
6	Etodesnitazene	12/14/2021	9 fatal overdoses and 1 non-fatal overdose
7	*N,N*-Dimethylpentylone	04/20/2022	26 fatal overdoses and 1 non-fatal overdose
8	Bromazolam	06/15/2022	236 fatal overdoses and 14 non-fatal overdoses

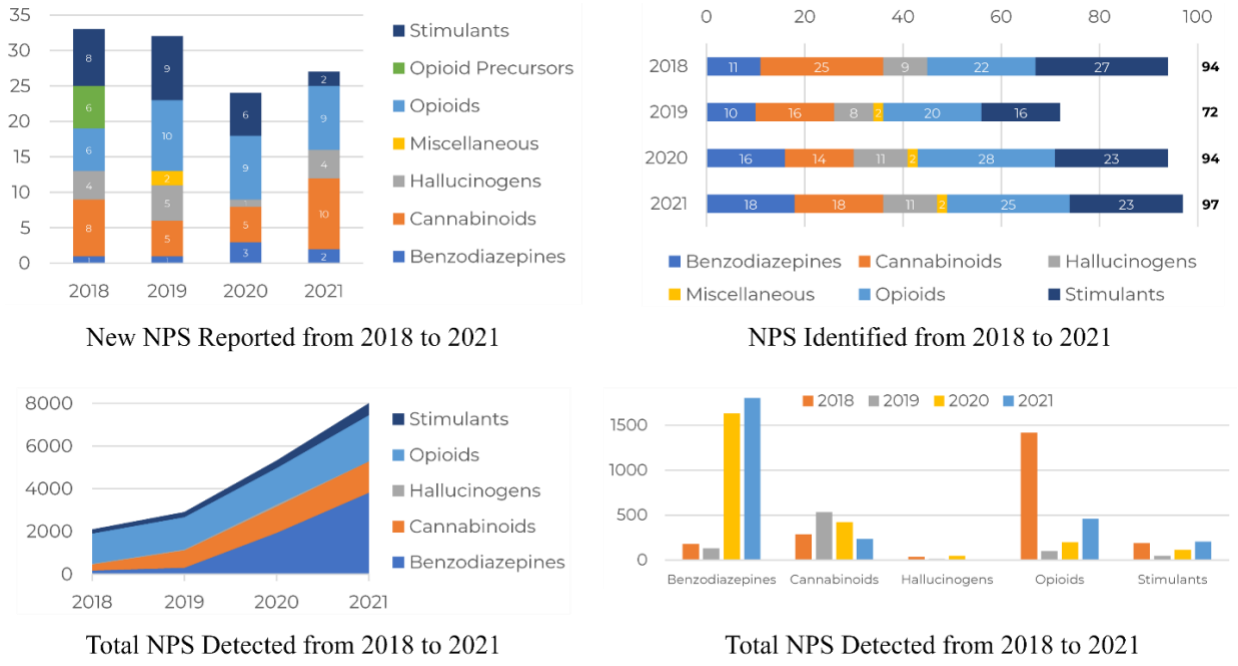

Figure 5: Figures from 2021 *Year in Review* depicting the emergence of NPS by subclass (https://www.cfsre.org/nps-discovery/trend-reports).

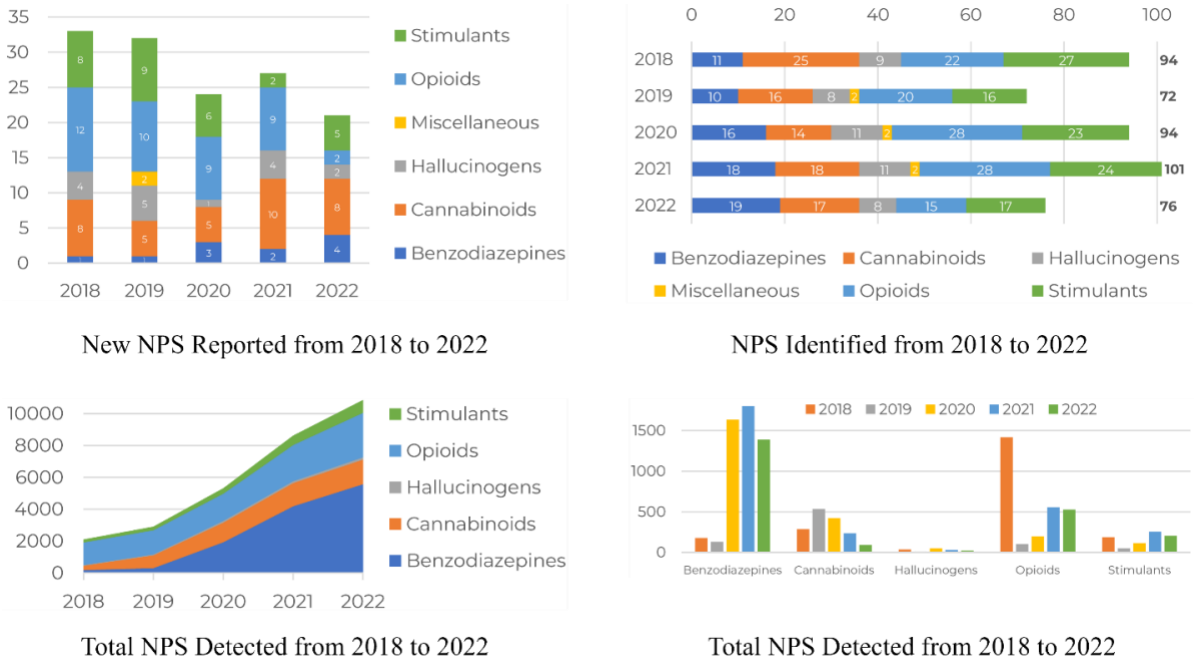

Figure 6: Figures from 2022 *Year in Review* depicting the emergence of NPS by subclass (https://www.cfsre.org/nps-discovery/trend-reports).

4.3 Objective 3: NPS Metabolism

The primary metabolites of four synthetic cannabinoids (ADB-4en-PINACA, ADB-FUBIATA, BZO-CHMOXIZID, CH-PIATA), two synthetic opioids (metonitazene, *N*-pyrrolidino etonitazene), one synthetic cathinone (*N,N*-dimethylpentylone), and one novel benzodiazepine (bromazolam) were investigated through *in vitro* experiments. The primary biotransformations for each investigated NPS were determined through analysis of authentic biological specimens, when possible (**Table 5**). Proposed metabolic schemes based on experimental analyses are shown in **Figures 7-14.**

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)	Product Ions
ADB-4en-PINACA	***Parent***	***$C_{19}H_{26}N_4O_2$***	***343.2131***	***0.6***	***298.1903, 213.1013, 326.1849, 145.0392***
	Amide hydrolysis + hydroxylation	$C_{19}H_{25}N_3O_4$	360.1915	-0.9	332.1970, 247.1077, 145.0381, 229.0971
	Hydroxylation	$C_{19}H_{26}N_4O_3$	359.2077	-0.1	314.1842, 229.0951, 342.1785, 145.0382
	Dihydroxylation	$C_{19}H_{26}N_4O_4$	375.2024	-0.7	330.1839, 245.0911
	Hydroxylation + internal hydrolysis	$C_{19}H_{28}N_4O_4$	377.2181	-0.6	332.1951, 247.1068, 360.1899, 145.0388
	N-dealkylation	$C_{14}H_{18}N_4O_2$	275.1499	-1.3	145.0369, 230.1295
	N-dealkylation	$C_{13}H_{15}N_3O$	230.1289	0.3	145.0387
	Amide hydrolysis	$C_{19}H_{25}N_3O_3$	344.1967	-0.4	213.1020, 298.1926

ADB-FUBIATA	***Parent***	***$C_{23}H_{26}FN_3O_2$***	***396.2090***	***2.00***	***238.1019, 86.095, 351.1862, 109.0437, 379.1809***
	Hydroxylation	$C_{23}H_{26}FN_3O_3$	412.2038	1.80	254.0979, 367.1821, 109.0442, 86.0956, 395.1767
	Hydroxylation	$C_{23}H_{26}FN_3O_3$	412.2039	1.80	254.0981, 367.1827, 86.0958, 109.0446, 395.1787
	N-demethylation	$C_{16}H_{21}N_3O_2$	288.1711	1.60	130.0639, 86.0954, 243.1481, 271.1429
	Hydroxylation	$C_{23}H_{26}FN_3O_3$	412.2038	1.70	254.0978, 367.1815, 86.0957, 109.0439, 395.1771
	Dihydroxylation	$C_{23}H_{26}FN_3O_4$	428.1987	1.6	270.0901, 383.1814
	Loss of NH +ketone formation	$C_{23}H_{23}FN_2O_3$	395.1774	2.1	254.0977, 109.0436, 86.0944, 367.183
	Loss of NH +ketone formation	$C_{23}H_{23}FN_2O_3$	395.1773	1.8	254.0989, 367.1811, 109.0447, 86.095
	N-dealkylation + Hydroxylation	$C_{16}H_{21}N_3O_3$	304.166	1.4	259.1466
	Loss of NH and *N*-dealkylation + dehydrogenation	$C_{16}H_{18}N_2O_2$	271.1444	1.2	130.0642, 86.096, 243.1470
	Loss of NH + demethylation	$C_{22}H_{23}FN_2O_2$	367.1826	2.6	254.1016, 109.0443
BZO-CHMOXIZID	***Parent***	***$C_{22}H_{23}N_3O_2$***	***362.1866***	***0.7***	***105.0325, 77.0378***
	Ketone formation	$C_{22}H_{21}N_3O_3$	376.1657	0.3	105.0327, 77.0372
	Ketone formation	$C_{22}H_{21}N_3O_3$	376.2654	-0.4	105.033, 77.0378
	N-dealkylation + Hydroxylation	$C_{15}H_{11}N_3O_3$	282.0875	0.7	105.0302
	Hydroxylation	$C_{22}H_{23}N_3O_3$	378.1812	0	105.0325, 77.0377, 174.0654, 256.1439
	Hydroxylation	$C_{22}H_{23}N_3O_3$	378.1811	-0.4	105.033, 360.1709, 256.1441, 174.0639, 77.0381
	Hydroxylation	$C_{22}H_{23}N_3O_3$	378.1817	1.3	105.0333, 256.1448, 77.0381, 174.0662
	Hydroxylation	$C_{22}H_{23}N_3O_3$	378.1813	0.2	105.0324, 256.1439, 174.0656, 77.0373, 360.1697
	Dihydroxylation	$C_{22}H_{23}N_3O_4$	394.1759	-0.6	105.0332, 77.0385, 254.1289, 376.1658, 272.1388
	Ketone formation	$C_{22}H_{21}N_3O_3$	376.1657	0.3	105.0327, 77.0372, 358.1571
	Dihydroxylation	$C_{22}H_{23}N_3O_4$	394.1763	0.5	105.0324, 254.1283, 376.1642, 174.0657, 272.1398
	Hydroxylation	$C_{22}H_{23}N_3O_3$	378.1811	-0.2	105.0326, 256.1439, 174.0656, 77.0372, 162.0672
CH-PIATA	***Parent***	***$C_{21}H_{30}N_2O$***	***327.2422***	***-2.6***	***83.0857, 100.1117, 130.0640, 144.0803, 200.1431, 245.1643***
	Oxidation [Tail] (e.g., 5OH-CH-PIATA)	$C_{21}H_{30}N_2O_2$	343.2364	-4.6	69.0701, 100.1122, 130.0654, 144.0793, 216.1375, 261.1591, 325.2272
	Oxidation [Core/Linker]	$C_{21}H_{30}N_2O_2$	343.2380	-0.1	100.1115, 160.0762, 216.1380, 240.2323, 261.1634
	Oxidation [Head]	$C_{21}H_{30}N_2O_2$	343.2367	-3.8	116.1051, 130.0646, 200.1440 , 225.1253, 240.2308
	Di-Oxidation [Core/Linker]	$C_{21}H_{30}N_2O_3$	359.2324	-1.5	100.1114, 134.0596, 162.0540, 218.1177, 232.1333, 259.1440
	Di-Oxidation [Core/Linker+Tail]	$C_{21}H_{30}N_2O_3$	359.2327	-0.5	100.1109 , 146.0590, 200.1070, 214.1228, 232.1337, 341.2214
	Carboxylation (CH-PIATA N-pentanoic acid)	$C_{21}H_{28}N_2O_3$	357.2172	-0.2	85.0651, 100.1128, 230.1182, 258.1104, 339.2050
	Loss of C5H10	$C_{16}H_{20}N_2O$	257.1651	0.9	55.0549, 100.1130, 130.0647, 175.0857
	Loss of C5H10 + Oxidation [Core]	$C_{16}H_{20}N_2O_2$	273.1599	0.6	100.1124, 128.0493, 146.0596, 173.0701
Metonitazene	***Parent***	***$C_{21}H_{26}N_4O_3$***	***383.2077***	***-0.2***	***100.1121, 72.0808***
	N-Dealkylation	$C_{19}H_{22}N_4O_3$	355.1765	0.2	284.1030, 72.0808
	N,N-Didealkylation	$C_{17}H_{18}N_4O_3$	327.1447	-1.5	284.1030, 44.0500
	O-Dealkylation	$C_{20}H_{24}N_4O_3$	369.1925	1.0	100.1121, 107.0497

	Nitro Reduction	$C_{21}H_{28}N_4O$	355.2336	-0.7	100.1121, 72.0808
N-Pyrrolidino Etonitazene	***Parent***	***$C_{22}H_{26}N_4O_3$***	***395.2084***	***1.7***	***98.0958, 135.0782***
	O-Dealkylation	$C_{20}H_{22}N_4O_3$	367.1768	0.9	98.0959, 56.0475
	Oxidation	$C_{22}H_{26}N_4O_4$	411.2023	-1.0	114.0908, 96.0765
	Loss of C4H6	$C_{18}H_{20}N_4O_3$	341.1610	0.6	298.1137, 252.1223
	Nitro Reduction (Suspected)	$C_{21}H_{28}N_4O$	N/A	N/A	98.0958, 135.0782
	Nitro Reduction + *O*-Dealkylation	$C_{20}H_{24}N_4O$	337.2022	-0.3	98.0948
N,N-Dimethylpentylone	***Parent***	***$C_{14}H_{19}NO_3$***	***250.1438***	***-***	***149.0239, 135.0446***
	Hydrogenation	$C_{14}H_{21}NO_3$	252.1594	-	149.0239, 135.0446
	N-Deethylation (Pentylone)	$C_{13}H_{17}NO_3$	236.1281	-	188.1073, 175.0647
	Demethylenation	$C_{13}H_{19}NO_3$	238.1438	-	137.0239, 121.0290
	Hydroxylation	$C_{14}H_{20}NO_4$	267.1465	-	149.0239, 135.0446
Bromazolam	***Parent***	***$C_{17}H_{13}BrN_4$***	***353.0401***	***1.3***	***249.9969, 274.1208, 326.0302***
	Oxidation [M+H]+	$C_{17}H_{13}BrN_4O$	369.0349	1.2	351.0222
	Oxidation [M+H]+	$C_{17}H_{13}BrN_4O$	369.0349	0.9	341.0139

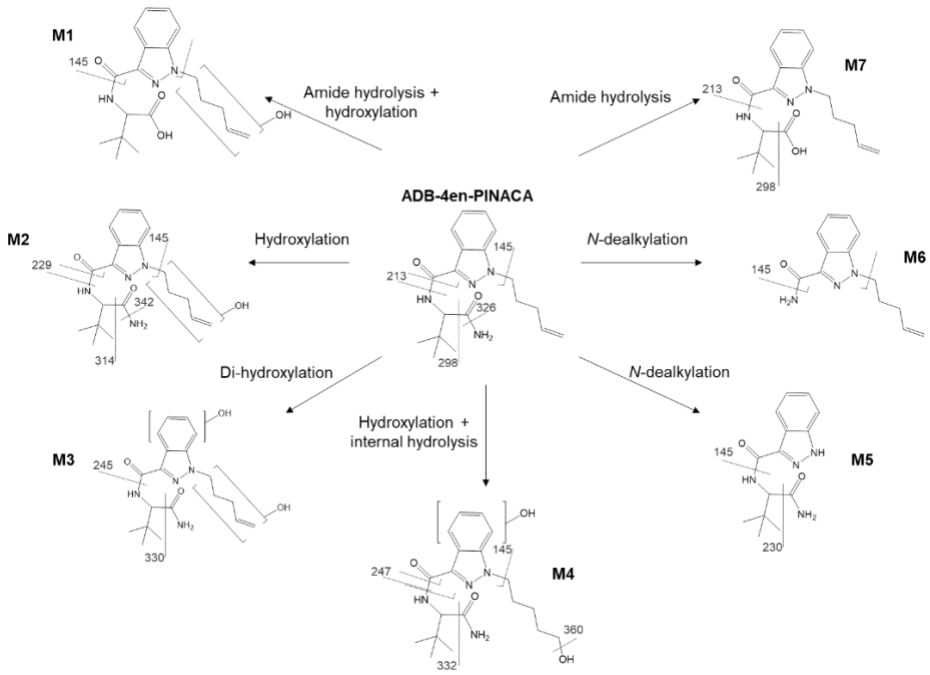

Figure 7: Proposed metabolism scheme for ADB-4en-PINACA (synthetic cannabinoid).

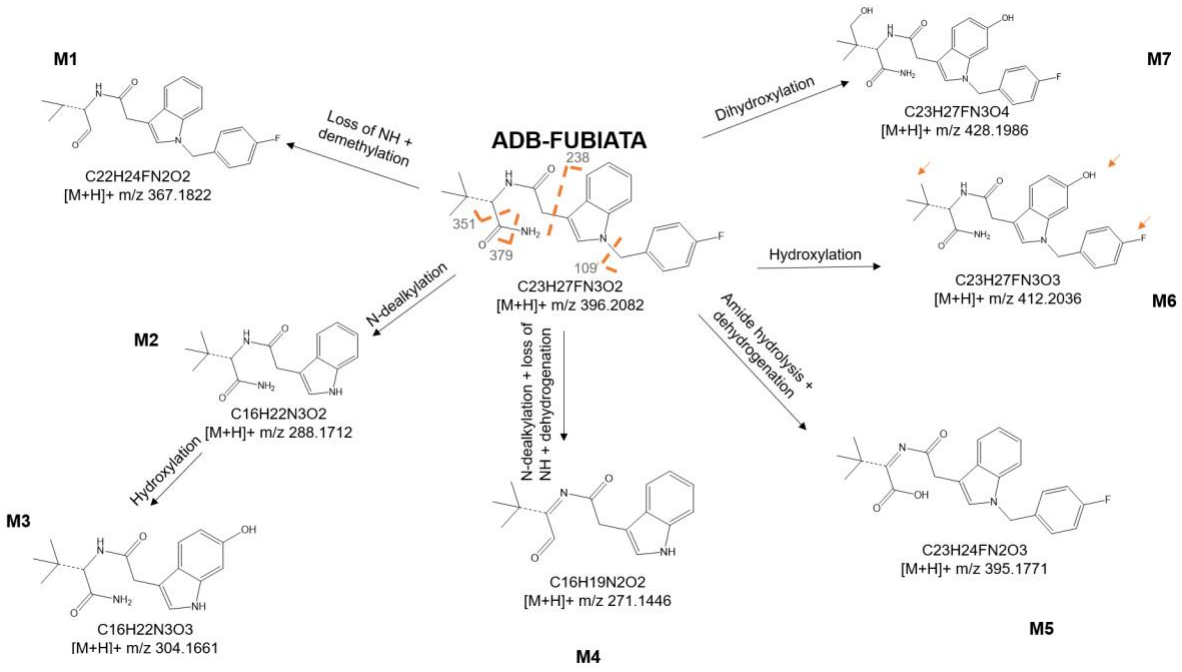

Figure 8: Proposed metabolism scheme for ADB-FUBIATA (synthetic cannabinoid).

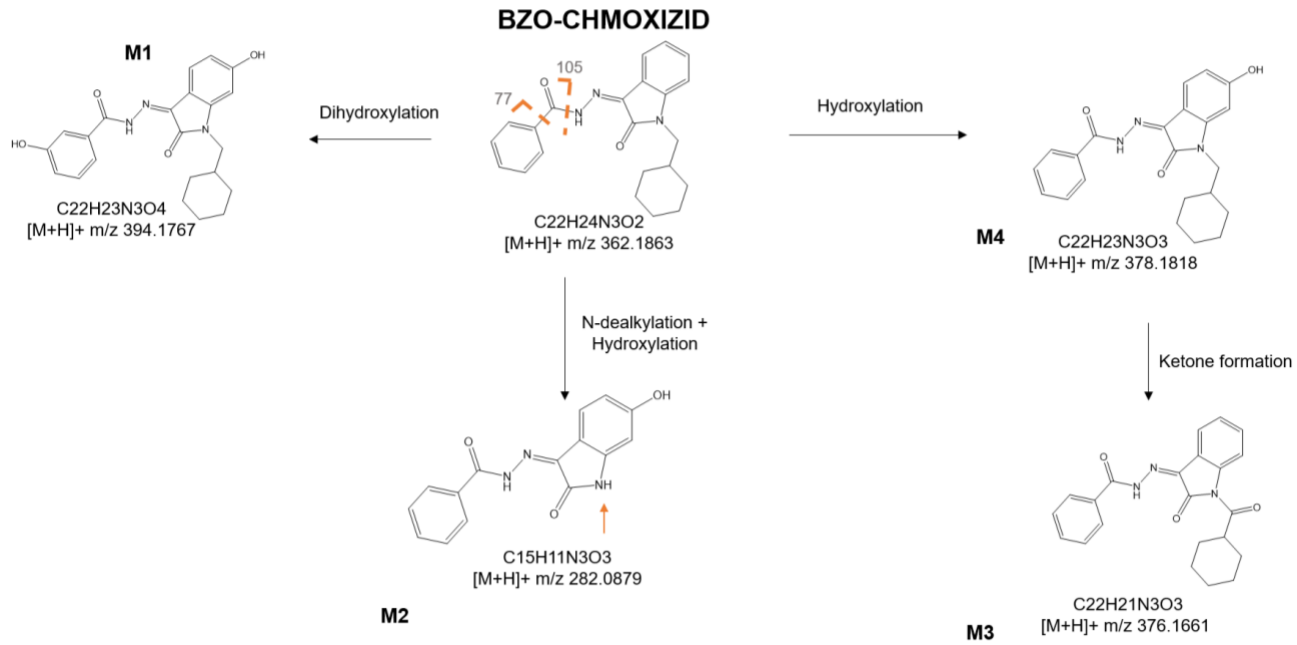

Figure 9: Proposed metabolism scheme for BZO-CHMOXIZID (synthetic cannabinoid).

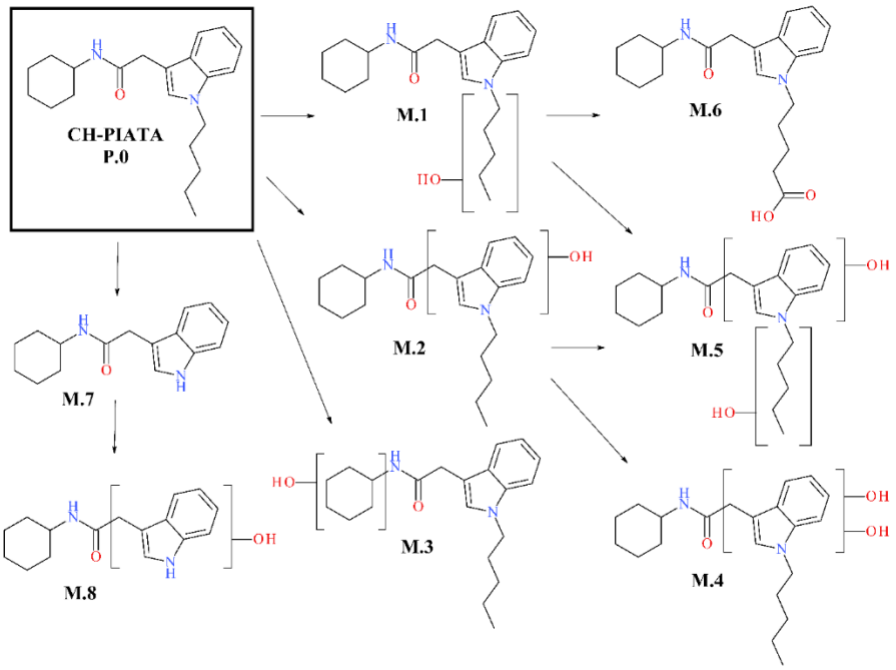

Figure 10: Proposed metabolism scheme for CH-PIATA (synthetic cannabinoid).

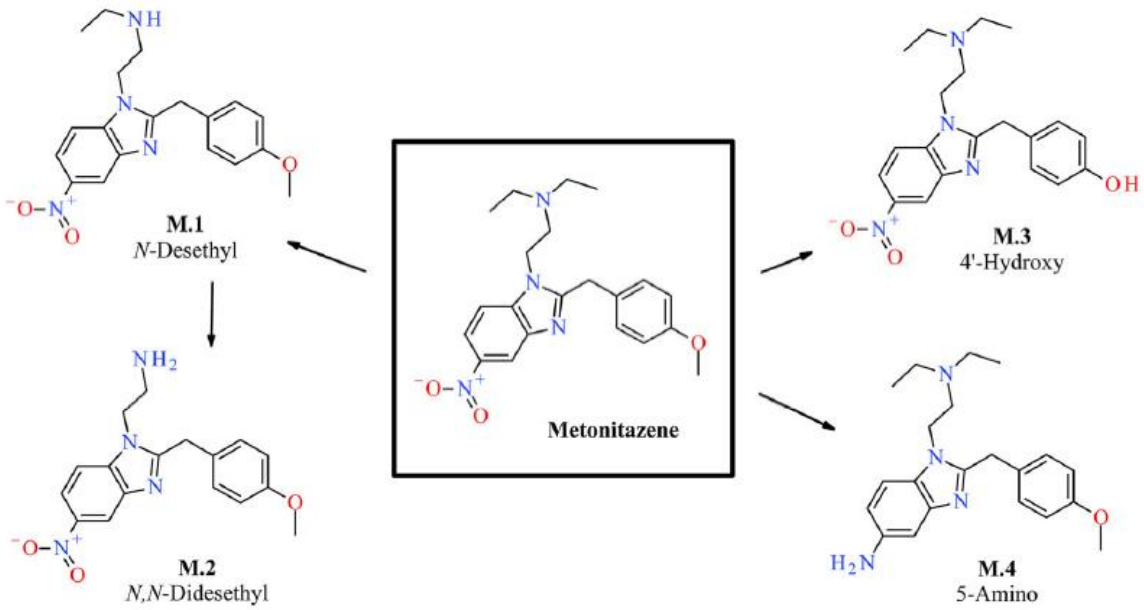

Figure 11: Proposed metabolism scheme for metonitazene (NPS opioid).

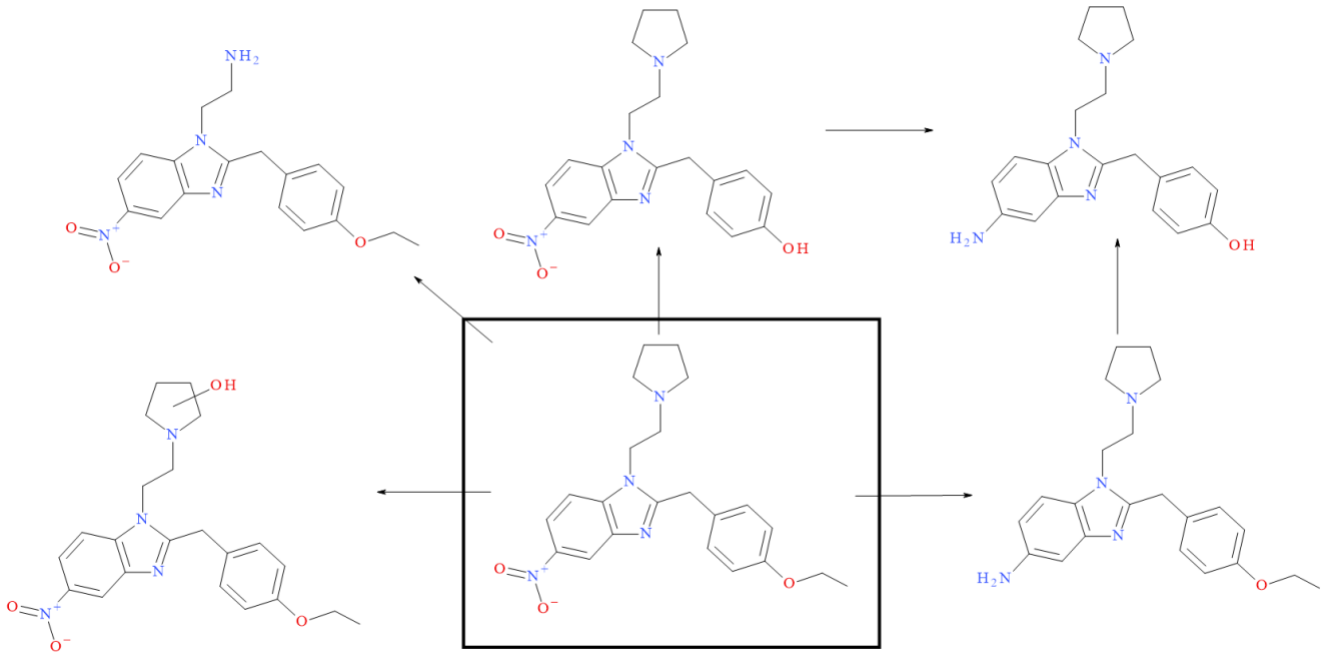

Figure 12: Proposed metabolism scheme for *N*-pyrrolidino etonitazene (NPS opioid).

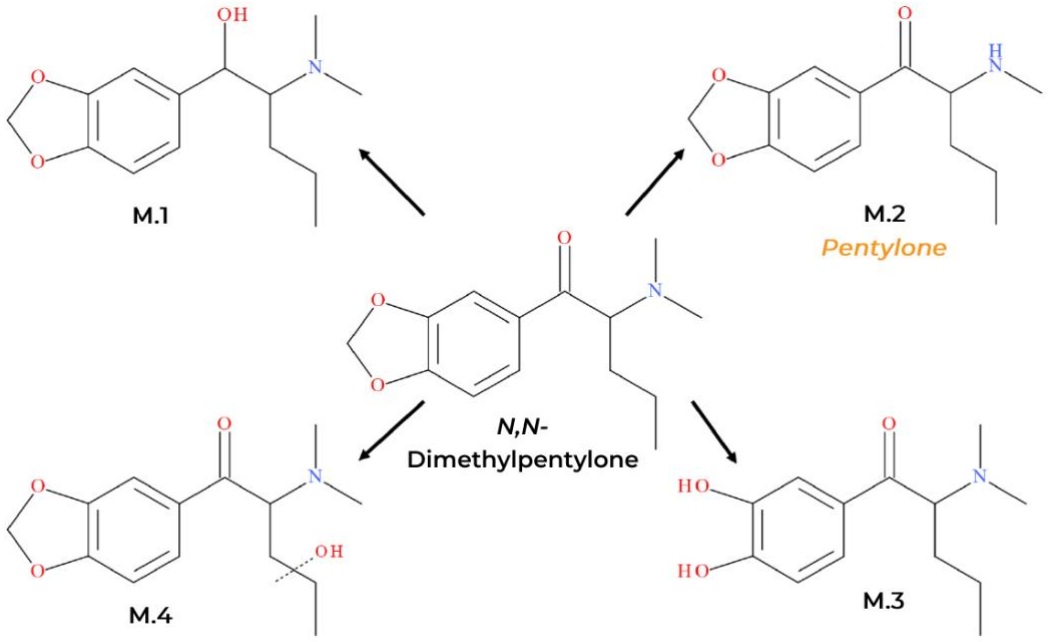

Figure 13: Proposed metabolism scheme for *N,N*-dimethylpentylone (NPS stimulant).

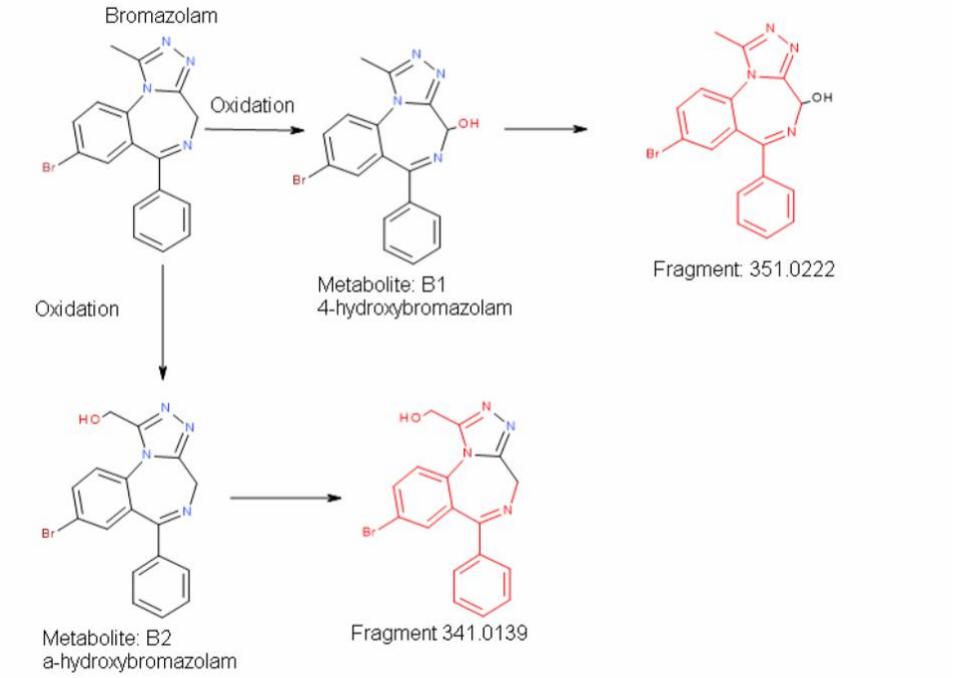

Figure 14: Proposed metabolism scheme for bromazolam (NPS benzodiazepine).

4.4 Objective 4: NPS Confirmation

Quantitative confirmations were performed for NPS to provide the forensic community with reference concentration data on these previously uncharacterized drugs. All methods were successfully developed and validated according to ASB Standard 036 and applied to authentic biological samples. Reference data acquired from these studies are shown in **Tables 6-9**.

Table 6: Reference concentration data for metonitazene in fatal overdoses compared to isotonitazene and brorphine (https://pubmed.ncbi.nlm.nih.gov/34137194/).

[ng/mL]	Isotonitazene		Brorphine		Metonitazene	
Matrix	**Blood**	**Urine**	**Blood**	**Urine**	**Blood**	**Urine**
N	18	18	18	18	21	15
Mean±Std. Dev.	2.2±2.1	2.4±1.4	2.5±3.1	4.6±7.6	6.3±7.5	14±13
Median	1.8	2.7	1.1	1.6	3.8	11
Min.	0.4	0.6	<0.1	0.2	<0.5	0.6
Max.	9.5	4	10	23	33	46

Table 7: Reference concentration data for a series of nitazene analogues in fatal and non-fatal overdoses (https://pubmed.ncbi.nlm.nih.gov/34792157/).

Case Type	Analyte	Matrix	N	Mean (±Std Dev)	Median	Min.	Max.
Postmortem	Isotonitazene	Central Blood	6	1.4 (±0.6)	1.5	0.6	2
		Peripheral Blood	10	2 (±2.5)	1.3	0.5	8.6
		Unspecified Blood	2	0.85 (±0.1)	0.85	0.8	0.9
		Urine	4	1.4 (±0.9)	1.2	0.6	2.5
		Bile	3	2 (±1.4)	1.6	0.9	3.5
	N-Desethyl Isotonitazene	Central Blood	1	3	N/A	N/A	N/A
		Peripheral Blood	3	1.1 (±1.0)	0.6	0.5	2.2
		Urine	6	4.5 (±6.1)	2.5	0.6	16
		Bile	3	90 (±39)	70	65	136
	Metonitazene	Central Blood	4	5.5 (±4.7)	4.2	1.6	12
		Peripheral Blood	16	5.5 (±3.5)	4.5	0.5	13
		Unspecified Blood	2	17 (±22)	17	1.4	33
		Serum	1	18	N/A	N/A	N/A
		Urine	12	14 (±13)	9.2	0.58	31
	Protonitazene	Central Blood	1	5	N/A	N/A	N/A
		Peripheral Blood	2	14 (±16)	14	3.1	25
		Urine	1	1	N/A	N/A	N/A
	Butonitazene	Unspecified Blood	1	3.2	N/A	N/A	N/A
		Serum	1	2.4	N/A	N/A	N/A
		Urine	1	10	N/A	N/A	N/A
	Etodesnitazene	Central Blood	1	30	N/A	N/A	N/A
	Flunitazene	Central Blood	2	2.7 (±2.9)	2.7	0.6	4.8
		Peripheral Blood	1	2.1	N/A	N/A	N/A
		Urine	1	0.5	N/A	N/A	N/A
	4'-Hydroxy nitazene	Urine	7	5.7 (±5.9)	2.9	0.7	14
		Bile	3	64 (±29)	52	43	98
DUID	Isotonitazene	Blood	50	1.6 (±1.9)	1	0.5	9
	N-Desethyl Isotonitazene	Blood	12	0.8 (±0.5)	0.6	0.5	2.2

Table 8: Reference concentration data for cinnamylpiperazines in fatal overdoses (https://pubmed.ncbi.nlm.nih.gov/35275255/).

Blood [ng/mL]	N	Mean	Std. Dev.	Median	Min.	Max.
2-Methyl AP-237	4	2280	2040	1250	820	5800
AP-238	2	-	-	-	87	270

Table 9: Reference concentration data for *N*-pyrrolidino etonitazene in fatal overdoses (https://pubmed.ncbi.nlm.nih.gov/35477798/).

***N*-Pyrrolidino Etonitazene in Blood (ng/mL)**					
N	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
21	2.5	1.9	2.2	0.3	25

4.5 Objective 5: NPS Toolkits

The creation of NPS toolkits allowed our laboratory to disseminate all technical scientific information regarding the chemistry and toxicology of emerging NPS in one report. Our NPS toolkits were developed to consolidate all information produced in our laboratory to allow stakeholders and collaborators to view and adapt this open-access information to their practicing laboratories. Our NPS toolkits contain analytical/instrumental data, mass spectra, analytical methods, drug intelligence, drug trends, drug combinations, outbreak data, and public alerts. Three NPS toolkits were released during this project period (**Table 10**).

Table 10: NPS Toolkits issued during the project period (https://www.cfsre.org/nps-discovery/analytical-toolkits).

#	Name	Date Released	Information Available
1	Metonitazene	07/21/2021	Public alert, trend plots, quarterly trend reports, new drug monograph, analytical methods, fatal concentrations, metabolism
2	*N*-Pyrrolidino Etonitazene	10/24/2022	Public alert, trend plots, quarterly trend reports, new drug monograph, analytical methods, fatal concentrations, metabolism
3	*N,N*-Dimethylpentylone	12/16/2022	Public alert, trend plots, quarterly trend reports, new drug monograph, analytical methods, fatal concentrations, isomeric relationships, metabolism

5. Applicability to Criminal Justice

This project directly addressed the need for improved identification and characterization of NPS, followed by rapid and accurate dissemination of information. Improved NPS surveillance and monitoring allowed new information to be circulated to key stakeholders, including law enforcement, federal entities, forensic scientists, and medical personnel. The project described allowed for the immediate addition of NPS to scopes of testing to allow for comprehensive analysis, as well as access to trend information for scientists to follow which drugs are increasing or decreasing in prevalence.

The identification and characterization of NPS trends by our laboratory provided reference concentrations for fatal and non-fatal overdoses as well as population impacts which in turn directly impacted drug legislation and scheduling actions (**Table 11**). Providing grant-funded comprehensive testing for NPS allowed for accurate reporting of NPS trends in fatalities and overdose outbreaks, which allows for accurate reporting of cause/manner of death and can direct proper treatment for non-fatal overdoses in clinical settings. This project generated up-to-date information on NPS which impacted laboratory, public health, and public safety systems.

Table 11: DEA drug scheduling actions impacted by CFSRE's NPS Discovery data.

Drug Name	Scheduling Action	Date	Link
Brorphine	Temporary Scheduling Order: Placement in Schedule I	03/01/2021	https://www.federalregister.gov/documents/2021/03/01/2021-04242/schedules-of-controlled-substances-temporary-placement-of-brorphine-in-schedule-i
Isotonitazene	Final Scheduling Order: Placement in Schedule I	11/04/2021	https://www.federalregister.gov/documents/2021/11/04/2021-23848/schedules-of-controlled-substances-placement-of-isotonitazene-in-schedule-i
Butonitazene	Temporary Scheduling Order: Placement in Schedule I	04/12/2022	https://www.federalregister.gov/documents/2022/04/12/2022-07640/schedules-of-controlled-substances-temporary-placement-of-butonitazene-etodesnitazene-flunitazene
Etodesnitazene			
Flunitazene			
Metodesnitazene			
Metonitazene			
N-Pyrrolidino Etonitazene			
Protonitazene			

PRODUCTS (ORIGINAL AWARD)

1. List of All Scholarly Products

The data generated as part of this research on NPS was consolidated into peer-reviewed publications and open-access reports for various stakeholders. This allowed for knowledge transfer from our laboratory to other experts in the field, such as forensic laboratories performing forensic casework, including medicolegal death investigations and DUID investigations. During this project period, seven peer-reviewed publications were published, and 15 conference presentations were presented. All the data gathered are housed at https://www.cfsre.org/nps-discovery.

1.1 Peer Reviewed Publications

- Alex J. Krotulski, Donna M. Papsun, Sara E. Walton, Barry K. Logan. Metonitazene in the United States – Forensic Toxicology Assessment of a Potent New Synthetic Opioid using Liquid Chromatography Mass Spectrometry. *Drug Testing and Analysis.* https://doi.org/10.1002/dta.3115
- Sara E. Walton, Alex J. Krotulski, Barry K. Logan. A Forward-Thinking Approach to Addressing the New Synthetic Opioid 2-Benzylbenzimidazole Nitazene Analogues by Liquid Chromatography Tandem Quadrupole Mass Spectrometry (LC-QQQ-MS). *Journal of Analytical Toxicology*. https://doi.org/10.1093/jat/bkab117
- Alex J. Krotulski, Nancy Garibay, Donna Walther, Sara E. Walton, Amanda L.A. Mohr, Barry K. Logan, Michael H. Baumann. Pharmacokinetics and pharmacodynamics of the synthetic cannabinoid, 5F-MDMB-PICA, in male rats. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2021.108800
- Melissa F. Fogarty, Marthe M. Vandeputte, Alex J. Krotulski, Donna M. Papsun, Sara E. Walton, Christophe P. Stove, Barry K. Logan. Toxicological and pharmacological characterization of novel cinnamylpiperazine synthetic opioids in humans and in vitro including 2-methyl AP-237 and AP-238. *Archives of Toxicology*. https://doi.org/10.1007/s00204-022-03257-7

- Marthe M. Vandeputte, Alex J. Krotulski, Donna Walther, Grant C Glatfelter, Donna M. Papsun, Sara E. Walton, Barry K. Logan, Michael H. Baumann. Pharmacological evaluation and forensic case series of N-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole 'nitazene' synthetic opioid. *Archives of Toxicology*. https://doi.org/10.1007/s00204-022-03276-4
- Sara E. Walton, Alex J. Krotulski, Grant C. Glatfelter, Donna Walther, Barry K. Logan, Michael H. Baumann. Plasma Pharmacokinetics and Pharmacodynamic Effects on the 2-Benzylbenzimidazole Synthetic Opioid, Isotonitazene, in Male Rats. *Psychopharmacology*. https://doi.org/10.1007/s00213-022-06292-5
- Donna M. Papsun, Alex J. Krotulski, Barry K. Logan. Proliferation of Novel Synthetic Opioids in Postmortem Investigations After Core-Structure Scheduling for Fentanyl-Related Substances. *American Journal of Forensic Medicine and Pathology*. https://doi.org/10.1097/paf.0000000000000787

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

One of the main objectives of this project was to rapidly disseminate information to law enforcement agencies, public health and safety agencies, forensic laboratories, criminal justice personnel, poison control centers, emergency departments, and medical examiner/coroner's offices, among other stakeholders. This was completed with email alerts from the NPS Discovery listserv, peer-reviewed publications, presentations at webinars or conference proceedings, and reports (e.g., new drug monographs, public alerts). One workshop presentation and six podcasts were presented during the project period.

2.1 Conference Presentations

- Impacts of an Early Warning System – The Tale of NPS Discovery. Alex J Krotulski*. Novel Synthetic Opioids in a Post Fentanyl Analog Environment, Workshop, American Academy of Forensic Sciences, Platform Presentation, February 2022.
- Monitoring Online Resources for Predicting Drug Threats. Alex J Krotulski*. Unforeseen Threats, Workshop, American Academy of Forensic Sciences, Platform Presentation, February 2022.
- An Explanation and the Impacts of a Class-Wide Ban on Synthetic Cannabinoids: What Does the Future Hold for This Already Challenging Novel Psychoactive Substances (NPS) Subclass? Alex J. Krotulski*, Judith Rodriguez Salas, Amanda L.A. Mohr, Barry K. Logan. American Academy of Forensic Sciences, Platform Presentation, February 2022.
- The Quantitation of a Synthetic Cannabinoid, 5F-MDMB-PICA, and Its Metabolites in Authentic Human Biological Samples Using Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS). Sara E. Walton*, Alex J. Krotulski, Donna M. Papsun, Barry K. Logan. American Academy of Forensic Sciences, Platform Presentation, February 2022.
- Developing Quarterly Scope Recommendations for Novel Psychoactive Substances (NPS) – A Multi-Disciplinary and Multi-Jurisdictional Approach to Assist Forensic Toxicology Laboratories. Alex J. Krotulski*, Donna M. Papsun, Dani M. Mata, Simon Elliott, Barry K. Logan. The International Association of Forensic Toxicologists, Platform Presentation, February 2022.
- NPS Discovery — Evolution of an open-access drug early-warning system. Alex J. Krotulski*, Barry K. Logan. Eighth International Conference on Novel Psychoactive Substances, Platform Presentation, November 2021.
- Examining the Evidence on Fluorofentanyl – Multidisciplinary Evaluation of this Emerging Drug with a Focus on Forensic Toxicology Investigations. Alex J. Krotulski*, Donna M. Papsun, Barry K. Logan. Society of Forensic Toxicologists (SOFT), Platform Presentation, September 2021.
- A Forward-Thinking Approach to Tackling New Synthetic Opioid "Nitazene" Analogues by Liquid Chromatography Mass Spectrometry. Sara E. Walton*, Alex J. Krotulski, Barry K. Logan. Society of Forensic Toxicologists (SOFT), Platform Presentation, September 2021.
- NPS Discovery: Evolution of Novel Synthetic Opioids in the United States with a Focus on Metonitazene. Barry K. Logan*, Donna M. Papsun, Sara E. Walton, Alex J. Krotulski*. Society of Forensic Toxicologists (SOFT), Platform Presentation, September 2021.
- Case Series involving Novel Cinnamylpiperazine Synthetic Opioids: 2-Methyl AP-237 and AP-238. Melissa F. Fogarty*, Alex J. Krotulski, Donna M. Papsun, Sara E. Walton, Barry K. Logan. Society of Forensic Toxicologists (SOFT), Platform Presentation, September 2021.
- NPS Discovery Toolkits: Real-Time Identification and Dissemination of Information Regarding Novel Psychoactive Substances. Sara E. Walton*, Alex J. Krotulski, Donna M. Papsun, Melissa F. Fogarty, Barry K. Logan. NIJ Symposium, March 2022.

- Benzo-Dope: An Increasingly Prevalent Drug Combination of Significant Toxicological Relevance. Krotulski AJ*, Papsun DM, Walton SE, Logan BK. Society of Forensic Toxicologists, Platform Presentation, November 2022.
- Pharmacology and Toxicology of N-Pyrrolidino Etonitazene – a New Nitazene Synthetic Opioid Increasingly Observed in Forensic Cases. Walton SE*, Vandeputte MM, Krotulski AJ, Walther D, Glatfelter GC, Papsun DM, Logan BK, Baumann MH, Stove CP. Society of Forensic Toxicologists, November 2022.
- Quantitation of the New Synthetic Cathinone N,N-Dimethylpentylone in a Post-Mortem Case Series. Fogarty MF*, Walton SE, Papsun DM, Lamb M, Logan BK, Krotulski AJ. Society of Forensic Toxicologists, November 2022.
- An Update: A Forward-Thinking Approach to Tackling New Synthetic Opioid Nitazene Analogues by LC-QQQ-MS. Walton SE*, Krotulski AJ, Logan BK. Society of Forensic Toxicologists, November 2022.

2.2 Webinars and Workshops

- Synthetic Cannabinoids: NPS in Combination with Everything! Alex J. Krotulski*. SOFT Webinar, June 2022.

2.3 General Press, Podcasts, and Other Media

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

PROJECT SUMMARY (CONTINUATION AWARD)

1. Goals and Objectives

This research addressed the need to develop key resources for NPS as limited information is often available for these emerging drugs. During this award, we proposed the compilation of information regarding NPS and traditional drugs in a user-friendly portal. This included classification and subclassification of drugs with information regarding preferred and other names, chemical properties, pharmacological effects, literature patents, scientific articles, and more information as was available. This process allowed for the creation of standardized processes for drug nomenclature and taxonomy and housing for additional resources and products to aid forensic scientists, public health officials, and other stakeholders.

1.1 Purpose, Aims, and Objectives

The purpose of this research was to rapidly name and classify traditional drugs and NPS and to develop resources for use by practitioners dealing with these drugs, especially when limited information is available. For appropriate design and implementation, this research was divided into four key objectives:

1. **Expert Panel:** Gather an expert panel of scientists and professionals with a key knowledge base and experience with drug nomenclature, classification, and taxonomy.
2. **Data Collection, Triangulation, and Consolidation:** Conduct an extensive data collection effort to gather information about drugs, including NPS, including aspects such as names, synonyms, colloquial terms, chemical properties, pharmacological effects, etc. in resources such as peer reviewed literature, literature patents, scientific articles, trusted websites, etc. Using the data collected, develop data science tools for analysis and connection between the associated data points information, and develop manners in which that data can be collapsed and expanded.
3. **Database Development:** Populate an open-access database to house all the information for easy tracking and searching.
4. **Resources and Knowledge Transfer:** Consolidate research outcomes to generate, for example, documents, roadmaps, toolkits, guides, infographics, etc. detailing the processes and relevant information regarding drug nomenclature, classification, and taxonomy. Presentations at in person and on-line forensic science conferences were part of our dissemination strategy, as well as peer reviewed publication of key findings in appropriate forensic science journals.

The above model will provide an important avenue for knowledge creation and transfer from our laboratory to other forensic laboratories performing forensic casework and identifying unknown drugs with little available information. This approach strengthens the forensic science community by providing easy access to chemical and analytical resources.

2. Research Questions

2.1 Research & Development Needs

The OSAC for Forensic Science at NIST has published research and development needs for Chemistry: Seized Drugs & Toxicology (1). This guidance describes the need for research involving emerging drugs of abuse (i.e., NPS) and therapeutic agents. The committee endorses research that will allow for the development and optimization of novel techniques that can be used by the forensic toxicology community – while nomenclature and taxonomy are not analytical techniques used in the laboratory, they are important aspects that toxicologists and forensic scientists deal with daily.

The NIJ has investigated various forensic disciplines and published research and development solicitations. NIJ solicitation priorities include increasing the body of knowledge to guide and inform forensic science policy and practice and to produce useful materials or methods that have potential for forensic application. Research regarding NPS has been assessed as a major gap with limited research being conducted. This research project expressly addressed providing tools and workflows designed to produce accurate, timely, and useable information about emerging NPS that can guide and inform drug policy.

2.2 Knowledge Gaps

A primary knowledge gap for NPS relates to the true extent of involvement in forensic investigations and the total number of new drugs on the market at a given time. Neither can be assessed if experts cannot effectively communicate about these NPS. Current research and testing workflows do not allow for timely and accurate characterization of NPS in forensically relevant samples, leading to misidentification and under-reporting, as well as inappropriate reporting based on a wrong or uncommon name. When a new NPS emerges, there is a need to rapidly name the drug and then describe the chemical signature, activity, potency, toxicity, and other general information to aid both toxicological and drug chemistry applications, as well as criminal justice and legal aspects associated. Additional knowledge gaps of significance to forensic toxicology/chemistry relate to the epidemiology of a drug or NPS.

2.3 Importance of Research

This research fills a gap in knowledge critical to forensic science practitioners to ensure early discovery, naming, and classification of new drugs contributing to illicit drug trafficking, deaths, and injuries in the United States. As outlined by the White House's National Drug Control Strategy in May 2022 (13), the United States lacks a comprehensive national early warning system for new drug identifications that accurately and quickly creates alerts about the emergence and spread of new synthetic drugs for forensic, clinical, and regulatory purposes (although the report highlights the utility of the CFSRE's NPS Discovery program as a surveillance tool). 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 (2).

Many organizations need timely information on emerging drugs to alert their stakeholders; so, while the infrastructure exists to share information, it is frequently not timely or comprehensive, and in some cases may be inaccurate. At times, drug early warning systems or other entities will use different names for the same drug, which creates confusion and inaccurate classification or reporting. As NPS have approximately a 6- to 9-month life cycle (some a bit longer), immediate classification and taxonomy is critical. Drug nomenclature and taxonomy are not problems for the United States alone – this is a global problem for which the European Union Drugs Agency (EUDA) and the United Nations Office on Drugs and Crime (UNODC) can serve as key partners to assist with naming and classification, as well as dissemination.

2.4 Impacts

This project allowed experts to have proper data exchanges and communication about NPS and a more complete and common drug language. The work completed helped to raise awareness of emerging drug threats and NPS from the rapid development of preferred names, classifications, and subclassifications for NPS. The understanding and reporting of vital death statistics are rooted in accurate medicolegal death investigations, comprehensive forensic toxicology testing, and precise reporting, the latter of which is linked to drug nomenclature and taxonomy (i.e., drug names and classifications, respectively). Public health and safety officials agree that there is a need in the forensic science community for more systematic and streamlined naming conventions applied to various types of drugs, including recreational or illicit drugs (e.g., fentanyl, methamphetamine, cocaine), therapeutic drugs, and emerging drugs (e.g., NPS). This research developed, curated, and expanded a primary source of up-to-date information regarding drug nomenclature and taxonomy. This project directly impacted stakeholders by creating tools to help standardize drug names and classifications.

3. Summary of Project Design and Methods

3.1 Objective 1.1: Expert Panel

The intent of this objective was to gather experts in the field with strong chemistry backgrounds to tackle drug nomenclature, classification, and taxonomy. The proposed panel of expert scientists included people with extensive experience with naming drugs as knowledge about the history of drug nomenclature is necessary to classify new and emerging drugs. The expert panel was headed by the PI from the Center for Forensic Science Research and Education, alongside leading scientists from Cayman Chemical. Dr. Alex Krotulski (CFSRE) and Dr. Donna Iula (Cayman) specifically had the required expertise and experience to lead this expert panel, and the two have served for many years in roles related to this topic and on working groups with similar missions. The expert panel served as the key contributors to this work and was created to tackle tasks related to naming and classifying NPS.

3.2 Objective 1.2: Naming Documents

The intent of this objective was to assist forensic scientists, chemists, and others about the preferred naming of new and emerging NPS to clarify NPS identifications and reporting. The

leads of the expert panel were tasked with naming drugs, including rapidly naming emerging NPS. The expert panel developed workflows for how new NPS are named based on previous naming conventions and developed a review process for the names and classifications. Naming guidance documents were created to become a roadmap for nomenclature and classification, which was developed as a living document that can be constantly updated.

3.3 Objective 3: Database Development

The goal of this objective was largely to collect chemical data for drugs and NPS to create a warehouse of information that can be used by chemists, forensic scientists, and many others. Data collected included relevant data from peer-reviewed literature such as journal articles, patents, and other trusted sites. Other chemical information collected included (but was not limited to) name, synonyms, chemical structure, molecular formula, molecular weight, optical activity, defined stereocenters, charge states, SMILES string, InChI code and key, a description, CNS activity data, originator/inventor, patents, approval year, primary receptor targets, pharmacology, activity, potency, pharmacokinetics, dose forms, half-life, adverse events reports, toxicity data, drug concentrations, sourcing, publications, analytical data, common names, street names, classifications and subclassifications, identifiers, links to other sources (PubChem), related drugs, and more (as available). The data collected was evaluated to allow for appropriate consolidation of data such as linking drugs of similar classes, subclasses, and structure. The information warehouse was determined to be an online database that was developed by an outside contractor with input from the expert panel using the data collected.

4. Summary of Results

4.1 Objective 1.1: Expert Panel

During the project period, the PI determined the appropriate scientists for the expert panel, which includes many entities and agencies, including the CFSRE, Cayman Chemical, EUDA, UNODC, Canada Border Services Agency, Drug Enforcement Agency (DEA), Customs and Border Protection (CBP), Centers for Disease Controls (CDC), and others.

4.2 Objective 1.2: Naming Documents

Naming documents have been drafted, reviewed, and completed for NPS opioids and synthetic cannabinoids. The NPS opioids naming document focused on the most prevalent subclasses, including the nitazene analogues and fentanyl analogues (https://www.cfsre.org/nps-discovery/analytical-toolkits).The document includes information on the drug prototype for the subclass of interest (e.g., etonitazene for the nitazene analogues and fentanyl for the fentanyl analogues) and prefix and suffix modifications to indicate the change in chemical structure (**Figure 15-16**). The synthetic cannabinoid naming document focused on the semi-systematic alpha-numeric scheme for the naming of these drugs based on their structure (**Figure 17**). The semi-systematic naming scheme for synthetic cannabinoids is based on the four major groups of the structure: head, core, linker, and tail (https://www.cfsre.org/nps-discovery/analytical-toolkits).

- **Benzyl Group:** Modifications to the benzyl ring are named by adding a prefix in front of the root "nitazene" to reflect the nature of the substituent added.
  - **Examples:** Metonitazene, Isotonitazene, Clonitazene, Flunitazene, 4-Hydroxy Nitazene, Fluetonitazene, Methylenedioxynitazene, Ethyleneoxynitazene, Propylnitazene
- **Nitro Group:** Nitazene analogues lacking the 5-nitro moiety contain the word "des" prior to the "nitazene" root. Substitutions to the nitro group are then placed in front.
  - **Examples:** Etodesnitazene, Metodesnitazene, Clodesnitazene, 5-Methyl Etodesnitazene
- ***N,N*-Diethylamino Group:** Modifications to the *N,N*-diethylamino moiety are named to reflect that substituent added or removed and the component name is placed in front.
  - **Examples:** *N*-Desethyl Isotonitazene, *N*-Pyrrolidino Protonitazene, *N*-Piperidinyl Etonitazene, *N,N*-Dimethylamino Etonitazene, *N*-Pyrrolidino Etodesnitazene, *N*-Pyrrolidino Fluetonitazene

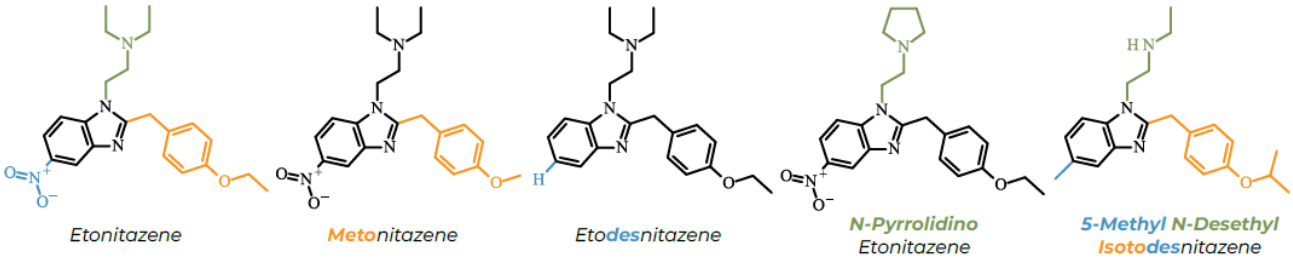

Figure 15: Examples of changes in nitazene analogue names based on structural subcomponent modifications (color).

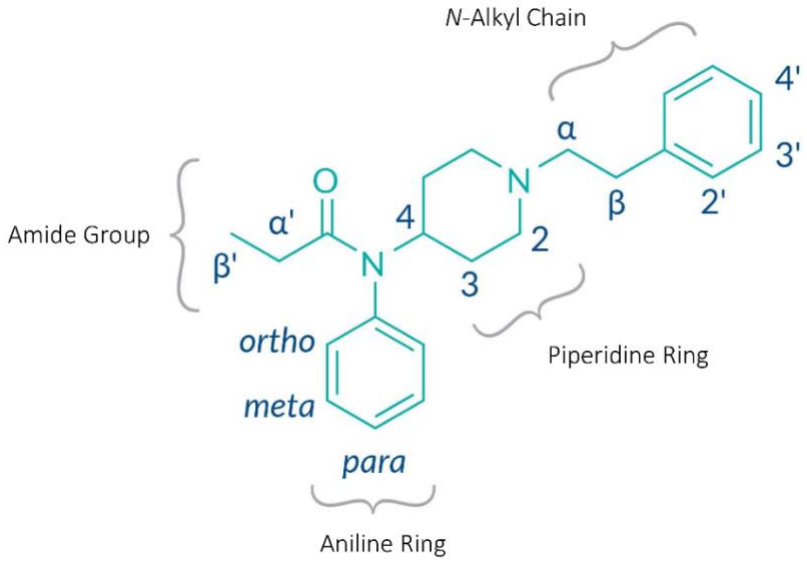

Figure 16: Core structure of fentanyl (left) with primary structural subcomponents showing the manner in which a new drug name changes based on the position of modifications or substitutions by using a semi-systematic approach (right). *[Source: Cayman Chemical]*

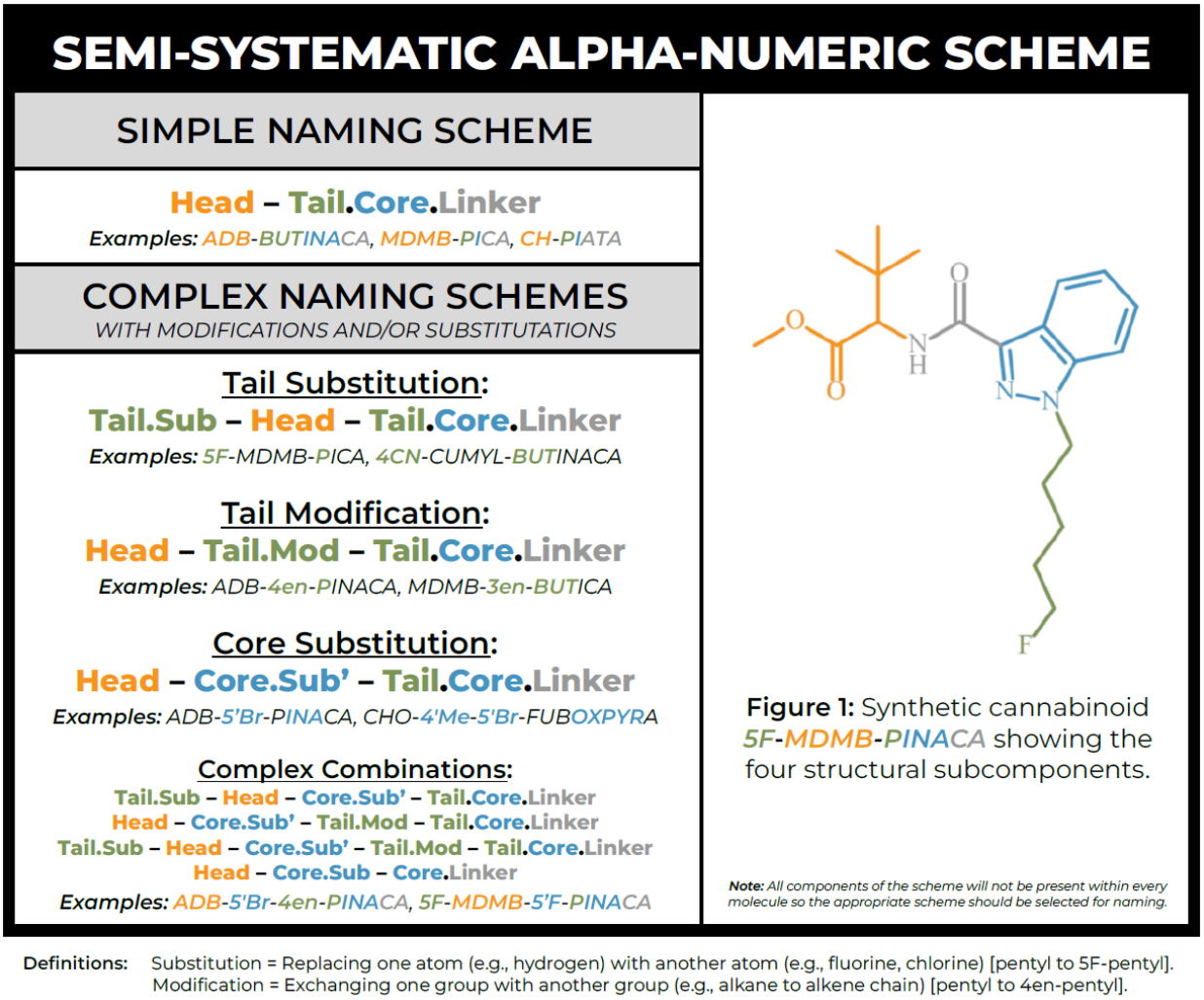

Figure 17: Depiction of the semi-systematic alpha-numeric scheme for the naming of synthetic cannabinoids.

4.3 Objective 3: Database Development

The Forensically Relevant Drug Database (FRDD) is under development and continued cultivation. The CFSRE requested quotes from consultants to build the FRDD and found that MRM Insights had the capability to build out the database to our specifications. MRM Insights was provided with a sample of the information necessary for each drug in the FRDD, which included the preferred drug name, synonyms, structure, formula, unique identifier, formal name (IUPAC), website links to appropriate literature, molecular mass, molecular ion, exact mass, SMILES, InChi strong, InChi key, CAS number, referent group, principal variant, T-code, biological effect, drug class, structural classification, origin/use, pharmacology, slang terms, drug type, control classification, and analytical data. The CFSRE will continually populate this database with drugs and NPS to create a one-stop-shop for information.

5. Applicability to Criminal Justice

This project directly addressed the need for improved communication between key stakeholders in the forensic community to allow for consistent and appropriate naming of drugs and NPS. The convening of the expert panel and the production of naming guidance documents allowed for large entities and agencies identifying NPS to come together to agree on approved naming of these complex drugs, which in turn allows for appropriate identification and consistent reporting of NPS. The development of an open-access database that will be continually updated to include new and emerging NPS allowed for a consistent stream of information to be provided to those interested in more information on NPS. Improved NPS naming and taxonomy allowed new information to be circulated to key stakeholders, including law enforcement, federal entities, forensic scientists, and medical personnel.

PRODUCTS (CONTINUATION)

1. List of All Scholarly Products

The data acquired from this project on NPS was consolidated into the database described as well as reports for various stakeholders such as forensic laboratories performing forensic casework, including medicolegal death investigations and DUID investigations. All the data gathered from this project are housed on the NPS Discovery website.

1.1 Peer Reviewed Publications

- N/A

1.2 Book Chapters, Theses, Conference Proceedings, etc.

- N/A

1.3 Technologies Developed (Patents, Prototypes, etc.)

- N/A

1.4 Software, Databases, Other Products

- Forensically Relevant Drugs Database (FRDD) – https://frdd.cfsre.org/

1.5 Archived Research Data

- CFSRE's NPS Discovery – https://www.cfsre.org/nps-discovery
- Naming Documents – 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

One of the main objectives of this project was to provide open-access information to law enforcement agencies, public health and safety agencies, forensic laboratories, criminal justice personnel, poison control centers, emergency departments, and medical examiner/coroner's offices, among other stakeholders. This was completed via conference presentations, webinars, and the development of the Forensically Relevant Drugs Database (https://frdd.cfsre.org/).

2.1 Conference presentations

- Developing an Approach to Standardize the Naming of Novel Psychoactive Substances (NPS). Alex J. Krotulski*, Sara E. Walton, Max Denn*, Brianna Stang, Barry K. Logan. NIJ Forensic Science R&D Symposium, American Academy of Forensic Sciences, Poster Presentation, February 2024.

2.2 Webinars and Workshops

- Robert Schelkun*, Alex Krotulski*. The Naming of Synthetic Cannabinoid Receptor Agonists Explained. NPS Discovery Webinar Series, October 2023. https://www.cfsre.org/resources/presentations/quarterly-nps-discovery-webinar-series-october-2023

2.3 General Press, Podcasts, and Other Media

- N/A

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