Document Title:	**Toxicological Time Travel: Retrospective Datamining of Analytical Time-of-Flight Mass Spectrometry (TOFMS) Data for Evaluating the Rise and Fall of Novel Opioid and Fentanyl Analog Use in the United States**
Author(s):	**Amanda LA Mohr, MSFS, D-ABFT-FT, Judith Rodriguez Salas, MS, Barry K Logan, Ph.D., F-ABFT**
Document Number:	**255883**
Date Received:	**December 2020**
Award Number:	**2017-DN-BX-0169**

Title:	**Toxicological Time Travel: Retrospective Datamining of Analytical Time-of-Flight Mass Spectrometry (TOFMS) Data for Evaluating the Rise and Fall of Novel Opioid and Fentanyl Analog Use in the United States**
Authors:	*Amanda LA Mohr, MSFS, D-ABFT-FT, Judith Rodriguez Salas, MS, and Barry K Logan, PhD, F-ABFT*
Organization:	*Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation, Willow Grove, PA, USA*
Award Number:	*2017-DN-BX-0169*

Abstract

Since 2013, the use of novel illicit opioids has been increasing. There are several new drugs and analogs of fentanyl that have emerged on the illicit drug market and account for many of the deaths that have occurred. There are challenges however in the timely identification of these new substances, and in alerting key stakeholders in public health and safety about the changes in the markets. The data are further limited by the lack of available reference standards, as well as the ability of overburdened crime laboratories and toxicology laboratories to develop and validate new methods, resulting in delays of months between the appearance of a new drug and the laboratories' ability to detect and report it. There is substantial variability between laboratories in terms of what is being tested for and reported. These limitations all impact the degree to which the data are actionable and help public health and safety agencies to intervene and reduce drug deaths.

To address these concerns, this project sought to data-mine raw electronic analytical data acquired using Liquid Chromatography Time of Flight Mass Spectrometry (LCTOFMS) from postmortem and driving under the influence of drugs (DUID) cases, and to find earlier and more timely identifications of new substances. The data-mining process, which involves repeated re-interrogation of the raw data against a continually updated database of emerging opioid drugs, has allowed the identification of key emerging opioids not included in the scope at the time of original analysis. From the data, time-course trend plots, geographic distribution heat maps, and basic demographic descriptions of populations dying from use of legacy opioids, and novel and emerging opioids were turned into reports. These reports were generated every three months, and disseminated within the public health, criminal justice, and forensic science communities to provide timely and updated information related to opioid trends in the United States.

Based on the application of the data-mining techniques developed under this award, throughout 2018 and into 2019 we were able to report within days of the close of each quarter that fentanyl positivity steadily increased, while heroin positivity remained relatively stable. Legacy prescription semi-synthetic opioids remained stable. The positivity for novel opioids in forensic cases significantly dropped in 2019, as scheduling of fentanyl analogs led to a reduction in their production and trafficking. By maintaining an updated and comprehensive scope, several emerging opioids were identified in 2018 and 2019. Once it became known that they were present in the United States, retrospective data-mining identified these emerging opioids in forensic toxicology casework performed during times when the compounds were not included in the scope of initial testing. This included 12 emerging opioids in 2018, and seven in 2019.

This research project resulted in the development of a real-time monitoring and early warning system for legacy and emerging opioid trends in the United States. Combining seized drug and analytical toxicological intelligence data, we were able to substantially reduce the lag time between new identifications of the drug in the street drug supply, and their detection in toxicological (postmortem and DUID) cases. On many occasions these new analytes were identified in cases several months prior to any awareness of their presence in the US drug market. The data demonstrate that opioid positivity has continued to increase throughout 2019, specifically for fentanyl, ahead of typical public health data systems, and that novel classes of emerging opioids have continued to appear on a recurring basis throughout the period of study supporting the proposal that resources should be allocated on an ongoing basis to support this successful approach to monitoring US drug markets.

1. Introduction

There are concerns from anecdotal reports from emergency room admissions, poison center calls, drug treatment admissions, drug possession and trafficking arrests, crime laboratory statistics, and medical examiner's data that the illicit use and abuse of both prescription opioids, such as morphine, fentanyl, and oxycodone, and traditionally abused opioids, most notably heroin, are increasing. The Centers for Disease Control (CDC) reported that since 1999 the number of synthetic opioid deaths have tripled (1, 2). Moreover, between 2014 to 2018 there was a steep increase in those numbers, reaching the highest mortality rates ever reported. In 2014, the CDC reported that more people died from drug overdoses than any other year on record, and the majority (60.9%) of those overdose deaths involved an opioid (3, 4). By 2018, there were more than 67,000 drug overdose deaths in the United States, with a 10% increase in synthetic opioid deaths from 2017 to 2018 (9% in 2017 to 9.9% in 2018) (3–6).

Beginning in 2013, novel opioid agonists became the next wave of the designer drug epidemic. Many novel opioid agonists that have been identified over the last seven years are now contributing to the opioid death statistics at an increasing, but poorly documented, rate (7). A study performed by Bowen et al. showed correlation between drugs mentioned in public drug-related forums and reported deaths for that specific drug (8). The increase in the number of posts for a specific compound, such as carfentanil, led to an increase in positivity in toxicological cases, just weeks after the postings.

These novel opioid agonists pose the same public health dangers as other novel psychoactive substance (NPS) classes including ease of accessibility over the internet, new drug introductions following scheduling, requirement for specialized toxicology testing, lack of certified reference material, limited knowledge of effects in humans, and misrepresentation to users. In addition to these challenges, there is also concern that these novel opioid agonists are

present in the routine illicit heroin and fentanyl drug supply, increasing the pool of potential victims. The market is complex with many novel drugs, and the information resources that allow public health and public safety agencies to assess the spread of new drugs as they enter the marker is significantly lacking. In efforts to regulate the rapid emergence of new synthetic opioids, the Drug Enforcement Administration (DEA) temporarily scheduled core structure fentanyl-related compounds, based on their structure and not on their toxicity/potency (9).

Traditional laboratory approaches to drug screening will typically fail to detect the novel opioids due to little or no cross-reactivity on traditional immunoassay tests (10). Some fentanyl derivatives do cross-react on some immunoassay platforms like enzyme-linked immunosorbent assay (ELISA), but if they are not in the scope of a confirmatory assay, they will result in screen positive results that fail to confirm (11). The most common screening approach is using gas chromatography mass spectrometry (GCMS); however, this technique lacks the necessary sensitivity required for detecting many NPS, and heavily relies on spectral libraries being regularly updated with the most current compounds. Many laboratories follow routine targeted testing for the presence of drugs but may not see compounds that are present outside of that scope. In many cases, standard reference material may not be available to confirm the identity of these compounds, and forensic laboratories that follow best practices and generally accepted accreditation standards will not report drugs as being present when they have not verified them against an authentic standard in their laboratory. Thus, many opportunities to identify new NPS early in their life cycle can be missed by limitations in the laboratory, and once missed in a case, their involvement will never be known unless the sample is retested, which occurs very infrequently for resource and cost reasons. Additionally, laboratory-to-laboratory variability in

terms of what drugs are tested for, and the small numbers and regional nature of cases processed by most laboratories, limits extrapolation and assessment of national novel opioid trends.

Further complicating the issue of drug toxicity is the presence of toxic, non-narcotic, adulterating substances in the street opioid supply that contribute to or complicate these intravenous drug deaths. Adulterants include levamisole, phenacetin, hydroxyzine, lidocaine, benzocaine, caffeine, acetaminophen, diltiazem, procaine, aminopyrine and prilocaine, in addition to sugars, bicarbonate, and starch (12–23). These substances can cause nausea, diarrhea, muscle pain, headache, fever, insomnia, dizziness, and convulsions. Potential complications associated with use of levamisole- and metamizole-laced cocaine include neutropenia, agranulocytosis, arthralgias, methemoglobinemia purpura retiform, systemic vasculitis, cutaneous necrosis, intravascular thrombosis, and skin necrosis (24–33). The chronic use of phenacetin is associated with nephrotoxicity leading to incontinence, back and flank pain, and can cause analgesic nephropathy, hemolytic anemia, methemoglobinemia, and kidney and bladder cancer (34). These substances are often overlooked, not tested for, and/or under-reported; however, the methodologies used for opioid drug testing are capable of detecting and reporting these drugs. In addition to their significance in drug-caused death, the identification of diluents and adulterants in toxicology specimens can have an important role in criminal investigations as they may be indicative of the drug sample origin, helping authorities identify the dealers and trafficking routes.

The net result of limitations in testing caused by policy, practices of testing, technology, and resources is that we currently have a very limited system across the United States for the timely identification of very toxic and dangerous drugs in the street drug supply. Currently, within the United States there is no national monitoring program to provide real-time clinical and

forensic toxicology data to medical, forensic, and law enforcement communities. Mortality data are limited in that these statistics are posted long after the end of the year at issue. Additional challenges stem from our limited ability to collect comprehensive data from various offices and collate that information in a timely manner into a system that accurately reports details of toxicologically confirmed deaths, along with the quantitative toxicology results that can allow sharing of information between states or between adjoining jurisdictions within the same state.

To address these limitations, the goal of this research project was to establish a pilot monitoring system using data processed with a systematic method and comprehensive but adaptable and evolving scope, collected from postmortem and impaired driving populations that would provide a unique window into the current landscape of the opioid epidemic, which is a critical public health and public safety issue. Additional objectives included data-mining for emerging opioids to track the change in positivity retrospectively and, in real-time, analyzing the data in relation to population demographics, and creating heat maps of drug positivity, all while rapidly sharing that information. This project also sought to collect data on toxic adulterants in opioid drug death and impairment cases to provide a more complete picture of the risks to drug users. Using this approach of "toxicological time travel" of being able to use knowledge developed today about emerging drugs to gauge their prevalence in the past, it was demonstrated that in many cases synthetic opioids and fentanyl analogs were implicated in cases previously tested, but in which opioid involvement was not known at the time of testing. Further, we postulated that as new NPS were identified, reprocessing of the archived data would lead to additional identifications in cases previously tested weeks or months prior.

2. Methods

2.1 TOF Data Acquisition

Data was acquired from driving under the influence of drugs (DUID) and postmortem (PM) cases submitted to NMS Labs (Horsham, PA) for analysis. NMS Labs is the largest reference laboratory for forensic toxicology testing in the United States, analyzing around 40% of all postmortem toxicology in the United States, including representative subpopulations of death investigations across the country. All samples were initially extracted using the same sample preparation procedures. Following extraction, extracts were analyzed using an Agilent Jet Stream 6230 time-of-flight mass spectrometer coupled to an Agilent 1290 liquid chromatograph (LC-TOF-MS, Santa Clara, CA). LC-TOF-MS generates high resolution mass spectrometry (HRMS) data that allows for exact mass determinations, which can aid in producing a chemical formula for unknown analytes.

Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 Rapid Resolution HT (3.0x100mmX1.8µm) column at 55°C with a flow rate of 0.7 mL/min with a total run time of 8.50 minutes. The mobile phases were 0.05% formic acid in 5 mM ammonium formate (A) and 0.05% formic acid in methanol (B). The gradient for the method is shown in Table 1.

Table 1. LC Gradient Conditions

Time	A	B
1.00 min	95.00%	5.00%
2.00 min	75.00%	25.00%
4.00 min	55.00%	45.00%
6.00 min	5.00%	95.00%
7.25 min	3.00%	97.00%
7.35 min	3.00%	97.00%
8.15 min	95.00%	5.00%
8.20 min	95.00%	5.00%
8.25 min	95.00%	5.00%

The number of datafiles varied between days, but approximately 100 samples including calibrators and controls were run per day on all four available instruments. Since all of the samples were prepared identically and acquired using the same instrumental parameters, data acquired on different instruments was equivalent and treated the same. The total number of samples analyzed ranged between 8,000 and 9,000 per month. Raw de-identified HRMS data acquired on each instrument was electronically transferred at the end of each day to computers at the CFSRE for further analysis.

Any sample that screened positive was subsequently sent for confirmatory testing prior to reporting. Reported data from NMS Labs was used to generate data for the legacy and novel opioids. The electronic data files were reprocessed using an expanded library to collect data on emerging opioids. A summary of the workflow is shown in Figure 1.

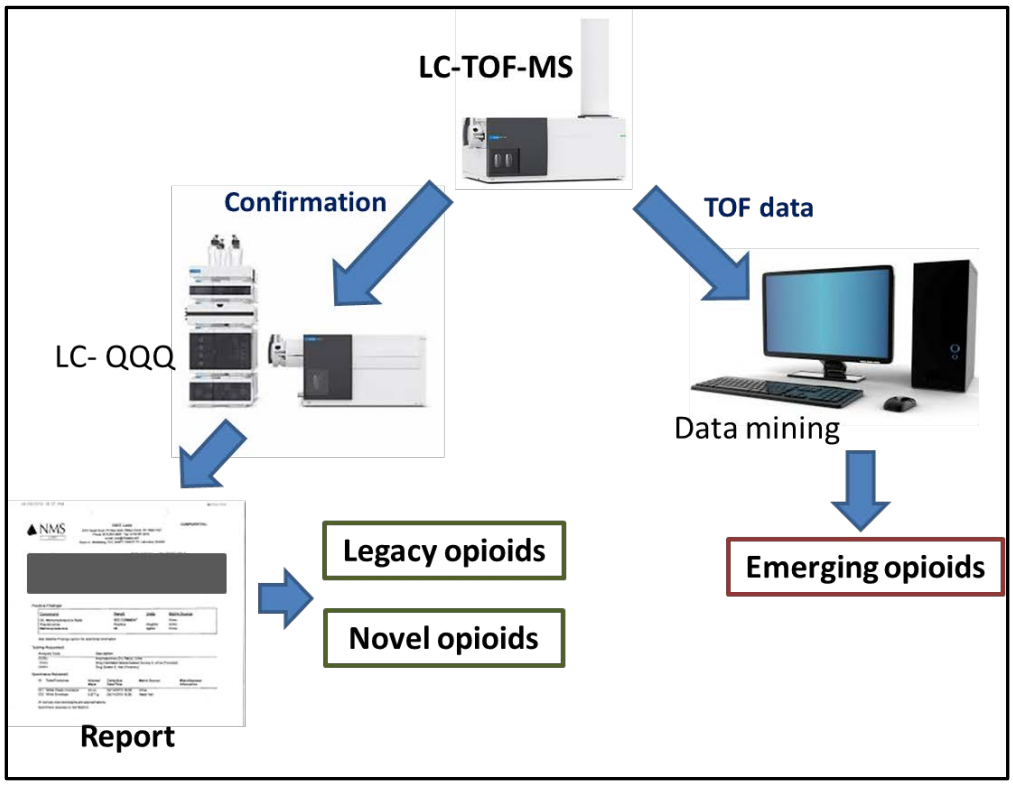

Figure 1. Data Collection Summary

2.2 Legacy Opioids

For the purposes of this report, legacy opioids were defined as morphine or morphine-like compounds, including prescription opioids and fentanyl that were implicated in forensic casework with some frequency prior to the onslaught of the opioid epidemic. A list of legacy opioids can be found Table 2.

Table 2. Legacy Opioids Include in the Scope of Testing

Legacy Opioid Analytes
Acetylfentanyl
Codeine
Dihydrocodeine/Hydrocodol
Fentanyl
Heroin (6-Monoacetylmorphine)
Hydrocodone
Hydromorphone
Methadone
Morphine
Oxycodone
Oxymorphone
Tramadol

To produce the trend reports, any legacy opioid reported by NMS Labs was tabulated along with basic demographic data, including age and sex, the state the sample originated from and type of agency submitting the case. Heroin positive cases were determined by detecting morphine in the blood and 6-monoacetylmorphine (6-MAM) in any other matrix associated with the case. It is important to note that the data has not been normalized to account for testing volume, and the geographical distribution is limited to the jurisdictions submitting samples for testing to NMS Labs.

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

2.3 Novel Opioids

Novel opioids were defined as compounds derived from fentanyl, its analogs, or other opioid analgesics pirated from pharmaceutical patents included in the initial scope of testing performed at NMS Labs. The scope for novel opioids can be found in Table 3.

Table 3. Novel Opioids Included in the Scope of Testing

Novel Opioid	Novel Opioid
(iso)butyryl-F-fentanyl N-benzyl analogue	Meta-Fluorofentanyl
3,4-Methylenedioxy U-47700	MT-45
4-Methoxybutyrylfentanyl	Ocfentanil
Acrylfentanyl	Ortho-fluorobutyrylfentanyl
AH-7921	Ortho-fluorofentanyl
Alfentanil	Para-fluorofentanyl
Alpha-methylacetylfentanyl	Papaverine
Alpha-methylfentanyl	Para-chlorofentanyl
Alpha-methylthiofentanyl	Para-chloroisobutyrylfentanyl
Benzodioxolefentanyl	Para-fluorobutyrylfentanyl/FIBF
Benzylfentanyl (R-4129)	Para-Methylfentanyl
Beta-hydroxyfentanyl	Para-Methylmethoxyacetylfentanyl
Beta-hydroxythiofentanyl	Para-fluoroacrylfentanyl
Beta-methylfentanyl	Phenylfentanyl
Butyrylfentanyl/Isobutyrylfentanyl	Remifentanyl
Carfentanil	Sufentanil
Cis/Trans 3-Methylfentanyl	Tetrahydrofuranfentanyl
Cis-3-Methylthiofentanyl	Thiofentanyl
Cyclopropyl/Crotonylfentanyl	Thiophenefentanyl
Cyclopentylfentanyl	Tianeptine
Desomorphine	U-47700
FIBF/Para-Fluorobutyrylfentanyl	U-48800/U-51754
Furanylfentanyl	U-49900
Furanylethylfentanyl	U-50488
Meta-Methylmethoxyacetylfentanyl	Valerylfentanyl
Methoxyacetylfentanyl (MAF)	W-15[1]
Meta-Fluorobutyrylfentanyl	W-18

[1] W-15 and W-18 have subsequently been shown not to have mu opioid agonist activity.

Data derived for the trend reports was compiled based on novel opioids reported by NMS Labs. In addition to the reported results, basic demographic data, including age and sex, the state the sample originated from, and the type of agency submitting the case were also collected. As with the legacy opioids, the data were not normalized to account for testing volume, and the geographical distribution is limited to the jurisdictions submitting samples for testing to NMS Labs.

2.4 Generation of Target Library

To be able to identify analytes in the datafiles acquired via LC-TOF-MS that were not included in the original scope of analysis, a more comprehensive library was created that contained new and emerging opioids in addition to legacy and novel opioid compounds. Available standards were purchased from Cayman Chemical (Ann Arbor, MI). Standards that came as a stock powder (1 mg) were prepared into a methanolic stock solution (1,000 ng/µL). To generate a retention time for each standard, neat standards were prepared (100 ng/mL) and analyzed on the LC-TOF-MS at NMS Labs.

Once the standard was run, the datafile was analyzed using the Agilent MassHunter Qualitative Navigator B.08.00 software (Santa Clara, CA). The formula of the standard was entered into the mass calculator in the software to obtain the exact mass with the H^+ ion species. Using the exact mass, an extracted ion chromatogram (EIC) was obtained, and the retention time was taken from the apex of the peak in the EIC. Subsequently, the Agilent Personal Compound Database and Libraries (PCDL) Manager was used to enter the name of the compound, the formula and the retention time of that analyte. Throughout the course of the project, various intelligence sources were monitored to identify emerging compounds. Once identified, if a

standard was available for the compound, it was purchased and added to the library as soon as possible with the aim of having a comprehensive, up-to-date database with the most current analytes available. In the event a standard was not available, the chemical formula and exact mass were added to the library. The following 120 analytes were added to the library database over the course of the project (Table 4).

Table 4. Emerging Opioids Added to the Library Data Base

Name	Formula	Exact mass	RT (min)	Date Added to Library
2,2,3,3-Tetramethyl-Cyclopropylfentanyl	C27H36N2O	404.28276	5.99	October 2018
2',5'-Dimethoxyfentanyl	C24H32N2O3	396.24129	5.297	August 2019
2'-Fluorofentanyl	C22H27FN2O	354.21074	5.113	October 2018
2'-Fluoro-ortho-fluoro (±)-cis-3-Methylfentanyl	C23H28F2N2O	386.21697	5.342	October 2018
2'-Fluoro-ortho-fluorofentanyl	C22H26F2N2O	372.20132	5.301	May 2019
2-Methyl AP-237	C18H26N2	270.2096	5.222	May 2019
3,4 Difluoro U-47700	C16H22F2N2O	296.17002	4.126	December 2019
3,4 Difluoro U-48800	C17H24F2N2O	310.18567	4.569	December 2019
3,4 Difluoro U-50488	C19H26F2N2O	336.20132	4.855	December 2019
3,4 Difluoro U-51754	C17H24F2N2O	310.18567	4.709	December 2019
3,4-Difluoro Isopropyl U-47700	C18H26F2N2O	324.20132	5.046	December 2019
3,4-Difluoro N-Desmethyl U-47700	C15H20F2N2O	282.15437	4.185	December 2019
3,4-Difluoro Propyl U-47700	C18H26F2N2O	324.20132	5.23	December 2019
3,4-Difluoro U-49900	C18H26F2N2O	324.2013	4.326	February 2020
3,4-Difluoro-N,N-Didesmethyl U-47700	C14H18F2N2O	268.1387	4.217	February 2020
3,4-Ethylenedioxy U-47700	C18H26N2O3	318.19434	3.869	August 2018
3,4-Ethylenedioxy U-51754	C19H28N2O3	332.20999	4.13	August 2018
3F-MT-45	C24H31FN2	366.24713	5.847	May 2019
4-ANBP	C18H22N2	266.1783	4.487	October 2018
4-Fluoro U-47931E	C15H21FN2O	264.16379	4.04	December 2019
4'-Methylacetylfentanyl	C22H28N2O	336.22016	5.018	August 2018
4'-Methylfentanyl	C23H30N2O	350.23581	5.339	August 2018
4-Phenyl U-51754	C23H30N2O	350.23581	5.654	August 2018
4-Phenylfentanyl	C28H32N2O	412.25146	5.7	October 2018
Alpha'-Methylbutyrylfentanyl	C24H32N2O	364.25146	5.547	August 2018
AP-237	C17H24N2O	272.18886	4.447	August 2019
Benzylfuranylfentanyl	C23H24N2O2	360.18378	4.905	October 2018
Bromadol (BDPC)	C22H28BrNO	401.13543	5.073	August 2018
Bromadoline (U-47931E)	C15H21BrN2O	324.08373	4.831	August 2018

Brorphine	C20H22BrN3O	399.09462	5.276	September 2019
Butorphanol	C21H29NO2	327.21983	4.715	September 2019
Cis-3-methylbutyrylfentanyl	C24H32N2O	364.25146	5.516	August 2018
Cyclobutylfentanyl	C24H30N2O	362.23581	5.549	August 2018
Cyclohexylfentanyl	C26H34N2O	390.26711	5.779	October 2018
Cyclopentenylfentanyl	C25H30N2O	374.23581	5.557	May 2019
Cyclopropaneacetylfentanyl	C24H30N2O	362.23581	5.37	August 2019
Despropionyl 2'-fluoro-ortho-fluorofentanyl	C19H22F2N2	316.1751	5.105	August 2018
Despropionyl Meta-methylfentanyl	C20H26N2	294.2096	5.206	October 2018
Despropionyl Ortho-(2)-fluorofentanyl	C19H23FN2	298.18453	4.98	October 2018
Despropionyl Ortho-methylfentanyl	C20H26N2	294.2096	5.172	August 2018
Ethoxyacetylfentanyl	C23H30N2O2	366.23073	4.815	August 2018
Ethyl U-47700	C17H24Cl2N2O	342.1266	5.46	February 2020
Fentanyl Methyl Carbamate	C21H26N2O2	338.19943	4.725	October 2018
Furanyl Norfentanyl	C16H18N2O2	270.13683	3.769	August 2018
Furanyl UF-17	C19H24N2O2	312.18378	4.812	May 2019
Hexanoylfentanyl	C25H34N2O	378.26711	5.818	October 2018
Isopropyl U-47700	C18H26Cl2N2O	356.14222	5.627	August 2018
Isotonitazene	C23H30N4O3	410.23179	5.656	November 2019
Isovaleryfentanyl	C24H32N2O	364.25146	5.563	August 2018
Meta-Methylfuranylfentanyl	C25H28N2O2	388.21508	5.301	October 2018
Metonitazene	C21H26N4O3	382.2005	4.906	February 2020
N-(2C-B) Fentanyl	C24H31BrN2O3	474.1518	5.217	December 2019
N-(2C-E) Fentanyl	C26H36N2O3	424.27259	5.799	December 2019
N-(2C-I) Fentanyl	C24H31IN2O3	522.13795	5.701	December 2019
N-(2C-N) Fentanyl	C24H31N3O5	441.22637	5.217	December 2019
N-(2C-P) Fentanyl	C27H38N2O3	438.28824	5.959	December 2019
N-(3-ethylindole) Norfentanyl	C24H29N3O	375.23106	5.143	October 2018
N-(DOBU) Fentanyl	C29H42N2O3	466.31954	6.138	December 2019
N-(DOM) Fentanyl	C26H36N2O3	424.27259	5.642	December 2019
N,N-Didesmethyl Loperamide	C27H29ClN2O2	448.1918	5.523	February 2020
N,N-Didesmethyl U-47700	C14H18Cl2N2O	300.07962	5.249	October 2018
N,N-Dimethylamido-despropionyl fentanyl (Urea fentanyl)	C22H29N3O	351.23106	5.129	October 2018
N-Benzyl para-fluoro-cyclopropyl norfentanyl	C22H25FN2O	352.19509	5.247	May 2019
N-Desmethyl U-47700	C15H20Cl2N2O	314.09527	5.206	October 2018
N-Methyl Norfentanyl	C15H22N2O	246.17321	3.735	August 2018
N-Methyl para-methylphenyl norfentanyl	C20H24N2O	308.18886	5.005	September 2019
N-Methyl U-47931E	C16H23BrN2O	338.09937	4.800	May 2019
N-Methylcarfentanil	C17H24N2O3	304.17869	4.043	October 2018
N-Methylcyclopropyl Norfentanyl	C16H22N2O	258.17321	4.073	October 2018

Norcarfentanyl	C16H22N2O3	290.16304	4.110	August 2018
Oliceridine	C22H30N2O2S	386.2028	5.418	October 2018
Ortho-Fluoroacrylfentanyl	C22H25FN2O	352.19509	4.941	October 2018
Ortho-Fluorofuranylfentanyl	C24H25FN2O2	392.19001	5.032	August 2018
Ortho-Isopropyl-furanylfentanyl	C27H32N2O2	416.24638	5.684	May 2019
Ortho-Methylacrylfentanyl	C23H28N2O	348.22016	5.275	May 2019
Ortho-Methylfentanyl	C23H30N2O	350.23581	5.282	October 2018
Ortho-Methylfuranylfentanyl	C25H28N2O2	388.21508	5.226	October 2018
Para-Bromo 4-ANPP	C19H23BrN2	358.10446	5.449	August 2019
Para-Bromo Fentanyl	C22H27BrN2O	414.13067	5.475	August 2019
Para-Chloroacetylfentanyl	C21H25ClN2O	356.16554	5.060	August 2019
Para-Chloroacrylfentanyl	C22H25ClN2O	368.16554	5.306	August 2018
Para-Chlorocyclopentylfentanyl	C25H31ClN2O	410.21249	5.888	October 2018
Para-Chlorocyclopropylfentanyl	C23H27ClN2O	382.18119	5.511	October 2018
Para-Chlorofuranylfentanyl	C24H25ClN2O2	408.16046	5.359	October 2018
Para-Chlorofuranylfentanyl 3-Furancarboxamide	C24H25ClN2O2	408.16046	5.433	August 2018
Para-Chlorovalerylfentanyl	C24H31ClN2O	398.21249	5.834	August 2018
Para-Fluoro-4-ANBP	C18H21FN2	284.16888	4.754	May 2019
Para-Fluoroacetylfentanyl	C21H25FN2O	340.19509	4.510	August 2018
Para-Fluorocrotonylfentanyl	C23H27FN2O	366.21074	5.240	August 2018
Para-Fluorocyclopropylfentanyl	C23H27FN2O	366.21074	5.209	August 2018
Para-FluoroFuranylfentanyl 3-Furancarboxamide	C24H25FN2O2	392.19001	5.223	September 2019
Para-Fluoromethoxyacetylfentanyl	C22H27FN2O2	370.20566	4.414	August 2018
Para-Fluorovalerylfentanyl	C24H31FN2O	382.24204	5.637	August 2018
Para-Methoxy-4-ANPP	C20H26N2O	310.20451	4.493	August 2019
Para-Methoxyacrylfentanyl	C23H28N2O2	364.21508	5.030	August 2018
Para-Methoxyfentanyl	C23H30N2O2	366.23073	5.130	August 2018
Para-Methoxyfuranylfentanyl	C25H28N2O3	404.20999	5.093	August 2018
Para-Methoxymethoxyacetylfentanyl	C23H30N2O3	382.22564	4.531	August 2018
Para-Methyl Isobutyrylfentanyl	C24H32N2O	364.25146	5.591	August 2018
Para-Methylbutyrylfentanyl	C24H32N2O	364.25146	5.616	October 2018
Para-Methylcyclopropylfentanyl	C24H30N2O	362.23581	5.493	August 2018
Para-Methylfuranylfentanyl	C25H28N2O2	388.21508	5.319	October 2018
Para-Methyltetrahydrofuranfentanyl	C25H32N2O2	392.24638	5.222	August 2018
Para-Toluoylfentanyl	C27H30N2O	398.23581	5.561	August 2019
Phenethyl 4-ANPP	C27H32N2	384.25655	6.084	December 2019
Phenylacetylfentanyl	C27H30N2O	398.23581	5.583	August 2018
Phenylbenzylfentanyl	C25H26N2O	370.20451	5.253	August 2018
Piperidylthiambutene	C17H21NS2	303.1115	5.428	September 2019
Pivaloylfentanyl	C24H32N2O	364.25146	5.573	August 2018

Propyl U-47700	C18H26Cl2N2O	356.14222	5.705	August 2018
Remifentanil Acid	C19H26N2O5	362.18417	4.341	October 2018
Tetrahydrothiophene Fentanyl	C24H30N2OS	394.20789	5.446	May 2019
U-47109 (Desmethyl U-47700 isomer)	C15H20Cl2N2O	314.09527	5.445	May 2019
U-48520	C16H23ClN2O	294.14989	4.616	May 2019
U-62066 (Spiradoline or U62)	C22H30Cl2N2O2	424.16843	5.55	October 2018
U-69593	C22H32N2O2	356.24638	4.544	August 2018
UF-17	C17H26N2O	274.20451	4.741	May 2019
β-Hydroxythioacetylfentanyl	C19H24N2O2S	344.15585	4.556	October 2018
β'-Methylcrotonylfentanyl (Senecioyl fentanyl)	C24H30N2O	362.23581	5.432	October 2018
β'-Phenylfentanyl	C28H32N2O	412.25146	5.739	October 2018

2.5 Emerging Opioids + Datamining

All LC-TOF-MS raw datafiles from 2018 and 2019 were transferred electronically to an HP Workstation Z240 – Core i7 computer. The datafiles were reprocessed using the Agilent MassHunter Qualitative Analysis Workflow B.08.00 software and processed using the "Find by Formula" method against the comprehensive library database. The parameters for the "Find by Formula" method are listed in Table 5.

Table 5. MassHunter Find by Formula Software Parameters

Software Parameters	Value
Formula matching	
Mass (ppm error)	± 20.00
Retention time	± 0.350 min
Ion Species	
Ion Species	H^+ and H^-
Scoring weight	
Mass	100
Isotope abundance	60
Isotope spacing	50
Retention time	100
Molecular confirmation/Low score matches	
Warn if score is	< 75.00
Do not match if	< 50.00

Following data processing, datafiles were reviewed using the following criteria in order to ascertain a presumptive positive finding (Table 6). If one or more of the criteria were not met, the result was subject to further review by the analyst.

Table 6. Data Processing Criteria

Reviewer Parameters	Value
Overall score	> 75.00
Retention time to library	± 0.100 min
Chromatography	Acceptable peak shape
Isotopic Pattern Score	> 50.00
Isotopic Abundance Score	> 50.00

Results were organized by score and flagged when the score was below 75 or when an analyte with multiple isomers was identified. When possible, the correct isomer was determined based on the closest match in retention time. If the determination could not be made, all isomers were reported. Samples identified as tentatively positive for emerging opioids were recorded into an Excel file with information related to the instrument, folder, datafile number, and sample identification number. A secondary review of the data was performed by a senior analyst, who evaluated the finding within the context of other positive findings along with additional review of the chromatography, mass spectra, and response.

2.6 Toxic Adulterants

All of the 2019 data that confirmed positive for one or more legacy opioids and/or novel opioids were analyzed for the presence of diltiazem, diphenhydramine, levamisole and/or xylazine in any matrix associated with the case. These analytes are common cutting agents, which are known to cause toxic effects within the human body. The data was evaluated by determining the percent positivity per analyte as well as by determining combinations of the toxic adulterants for heroin positive, fentanyl positive, heroin and fentanyl positive, and novel

opioid positive cases. For novel opioid cases, cases that were positive for more than one novel opioid were only counted once to avoid artificially inflating the number of cases present with toxic adulterants.

3. Results and Discussion

3.1 Legacy Opioids

Data related to the confirmation of legacy opioids in blood was collected for the second half of 2018 (June – December) and all of 2019. Figure 2 displays the number of positive cases by month for each of the legacy opioids in the scope for all 18-months of data. With respect to fentanyl, in the last six months of 2018 fentanyl positivity remained relatively stable. Beginning in 2019, there was an increase in fentanyl positivity in January with a decrease in February and a steady increase in positivity for the remainder of 2019. Like fentanyl positivity, heroin positivity remained relatively stable for the last six months of 2018 and into the beginning of 2019 before a dip in positivity in February 2019. Following February 2019, heroin positivity continued to increase, peaking in May 2019 with 619 positive heroin cases. Heroin positivity remained relatively stable for the remainder of the year. Morphine positivity followed similar trends to heroin for 2018 and 2019. From June 2018 into May 2019 acetylfentanyl positivity was steadily increasing; however, after May, acetylfentanyl positivity continued to decline for the rest of 2019. All other legacy opioids showed relatively stable trends in 2018 and 2019.

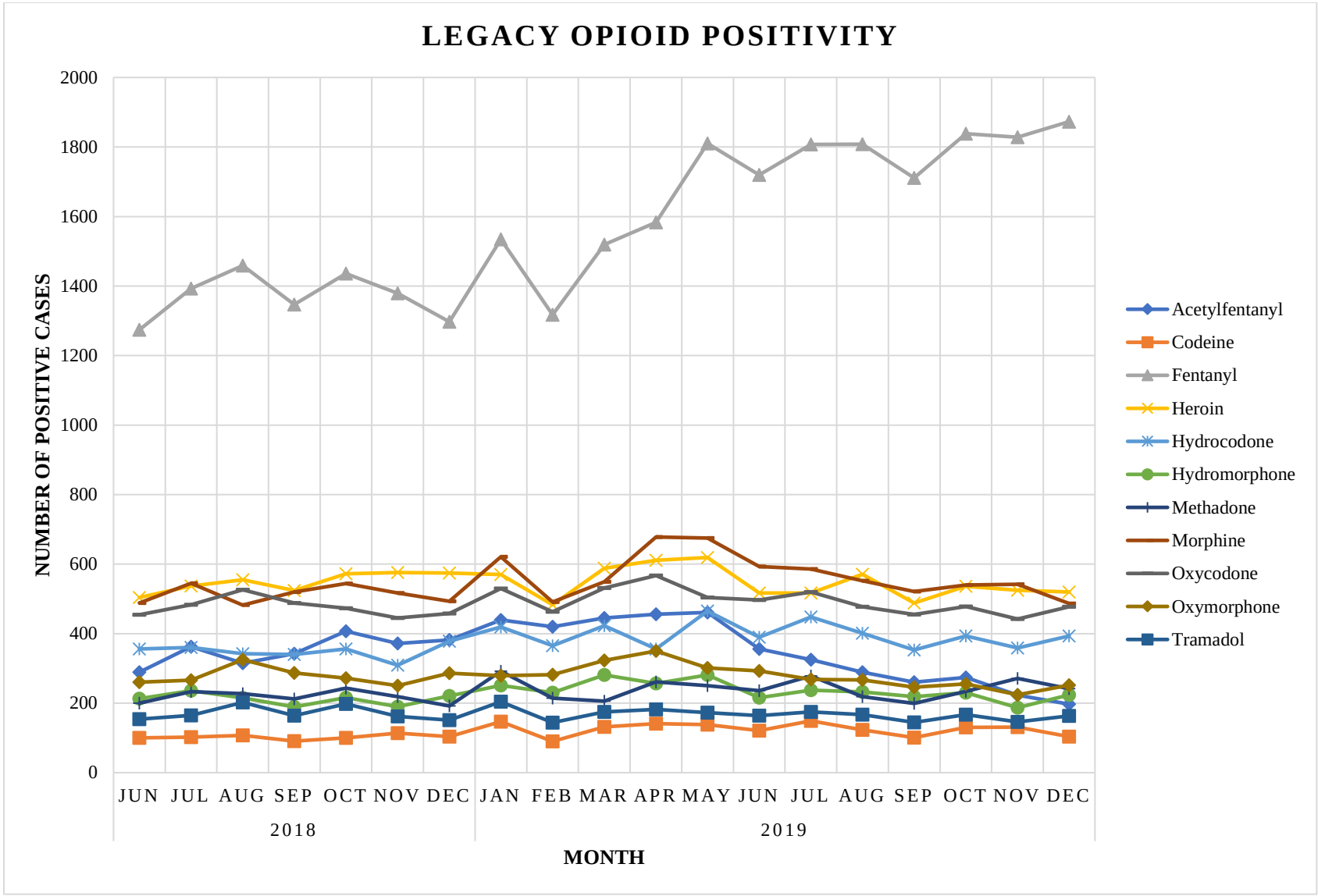

Figure 2. Legacy Opioid Positivity by Month

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

2018

Between June and December 2018, there were over 30,000 cases that confirmed positive for one or more legacy opioids during that time frame. Fentanyl accounted for the highest positivity with 9,585 cases followed by heroin with 3,842 and morphine with 3,589. In the six months of data for 2018, fentanyl and heroin cases showed steady rates of positivity with no significant increases or decreases during that time period. For fentanyl cases (n=9,585), there were 6,238 (65%) males and 2,371 (25%) females and 976 (10%) with an unknown sex. The mean and median age were 39 (±13) and 43, respectively, and the age range was 0-95 years old. Eighty-two percent (82%) of the cases were submitted by death investigators with 13% from law enforcement, 2% hospitals, and the remaining 3% from other agencies. A heat map of the 6-month positivity for fentanyl in the United States in 2018 is shown in Figure 3.

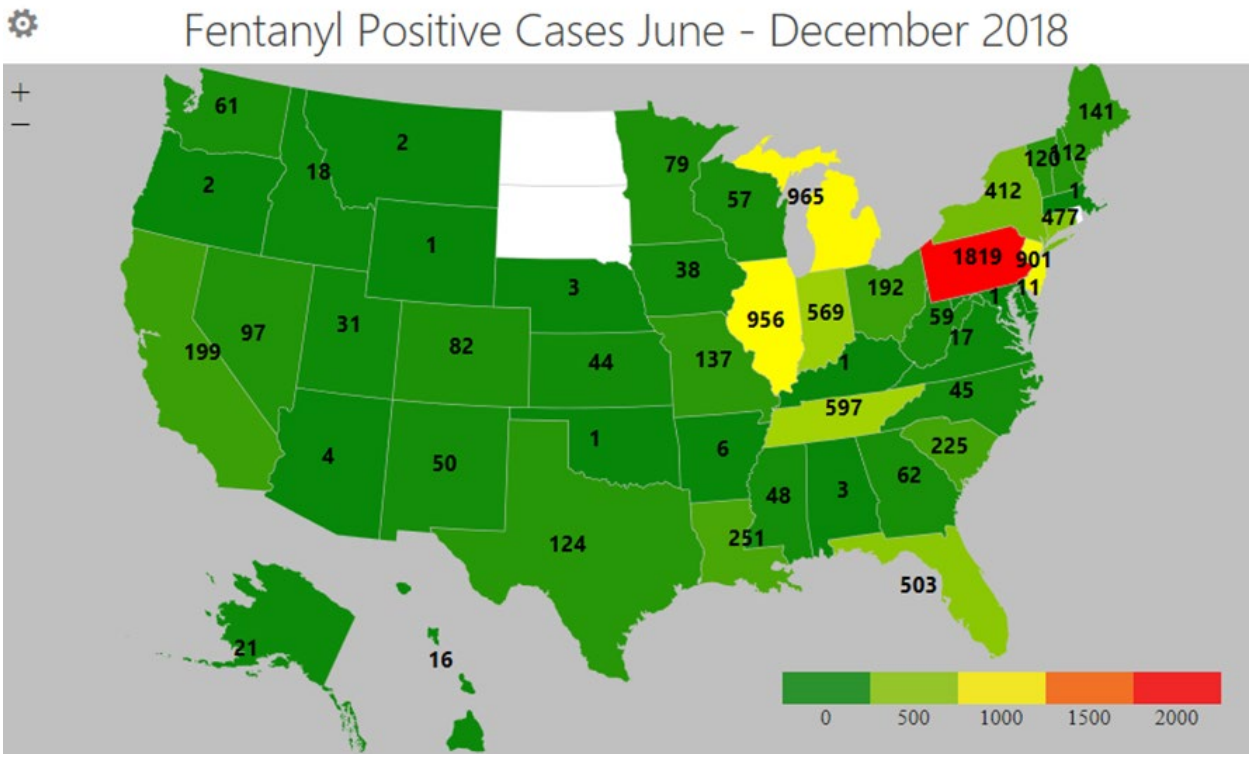

Figure 3. 2018 6-Month Geographical Distribution of Fentanyl Positive Cases

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

The number of heroin positive cases were 3,842 between June and December 2018. For the heroin positive cases, 2,694 (70%) were male, 846 (22%) were female, and 302 (8%) cases had an unknown sex. The mean and median age were 39 (±12) and 43, respectively, with an age range of 1-80 years old. With respect to case type, 95% of heroin positive cases were submitted by death investigators, 3% were from law enforcement, and the remaining 2% were submitted by other agencies. A heat map for the 6-month positivity for heroin in 2018 in the United States is shown in Figure 4.

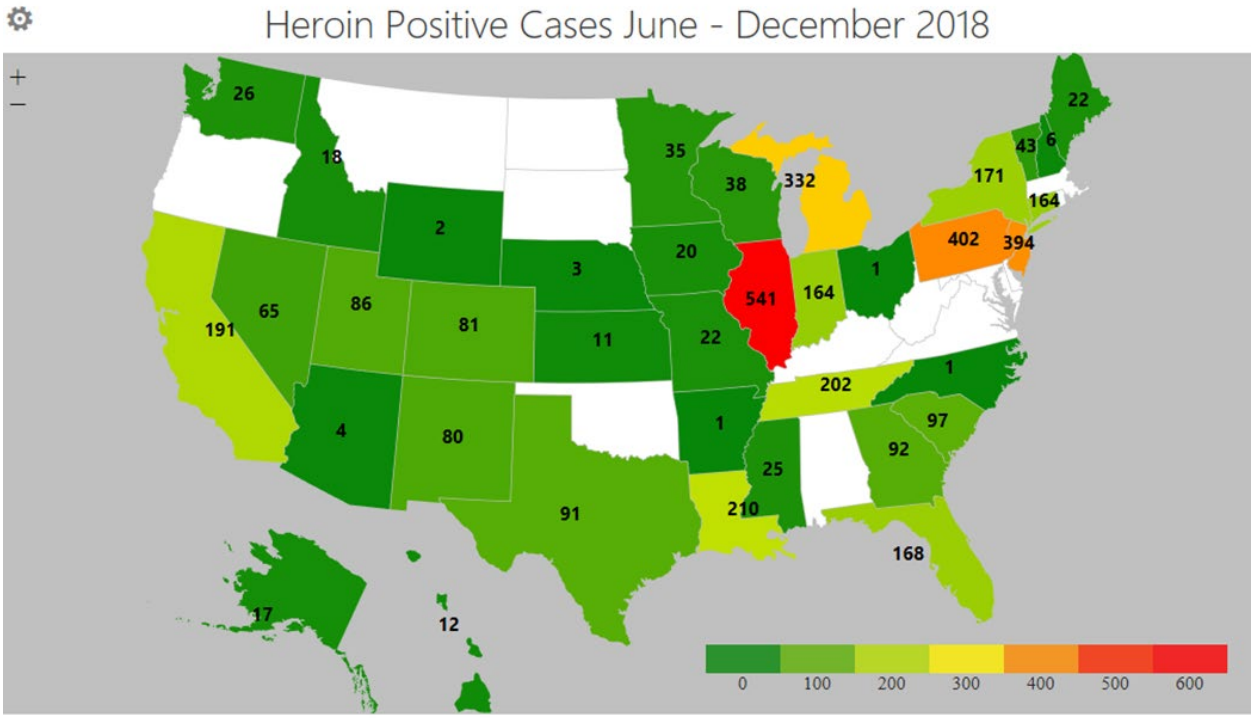

Figure 4. 2018 6-Month Geographical Distribution of Heroin Positive Cases

2019

In 2019, there were over 60,000 cases that were positive for one or more legacy opioid, which included 20,348 fentanyl positive cases and 6,545 heroin positive cases. With respect to the demographics associated with the fentanyl positive cases (n=20,348), 12,233 (60%) were

male, 4,833 (24%) were female, and the sex was unknown in 3,282 (16%) cases. The mean and median age were 40 (±12) and 43, respectively, with an age range of 0-109. The large majority of fentanyl cases were submitted by death investigators (84%) with 15% submitted by law enforcement agencies and 1% by hospitals. The geographic distribution of the fentanyl cases across the United States is shown in Figure 5. One anomaly between 2018 and 2019 positivity data for fentanyl was the addition of Maricopa County (suburban Phoenix) as client to NMS Labs in March 2019.

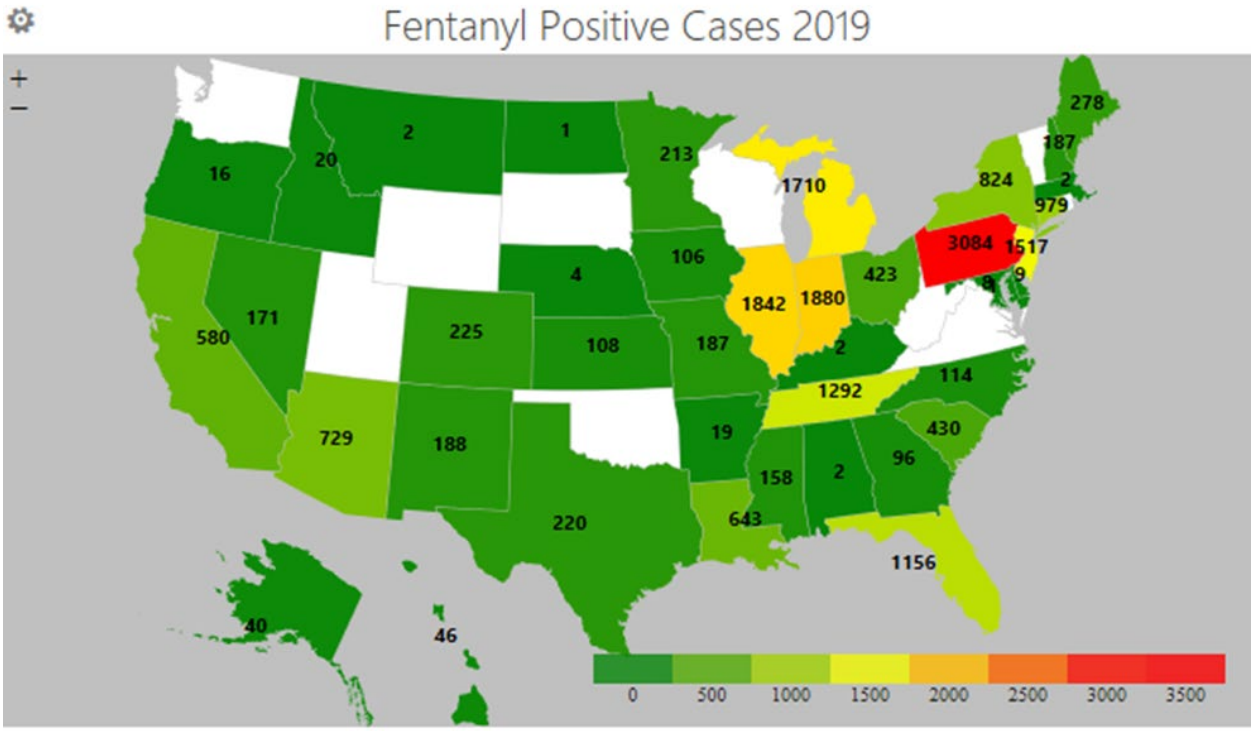

Figure 5. 2019 Geographical Distribution of Fentanyl Positive Cases

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

For heroin positive cases (n=6,545), a total of 4,444 (68%) were males, 1,449 (22%) were females, and 652 (10%) were positive cases where the sex was unknown. The mean and median age were 40 (±12) and 41, respectively with an age range of 0-86. Ninety-two percent (92%) of the cases originated from death investigators with 5% coming from law enforcement agencies. The geographic distribution of heroin cases in 2019 across the United States is shown in Figure 6. One anomaly between 2018 and 2019 positivity data for heroin was the addition of Maricopa County (suburban Phoenix) as client to NMS Labs in March 2019.

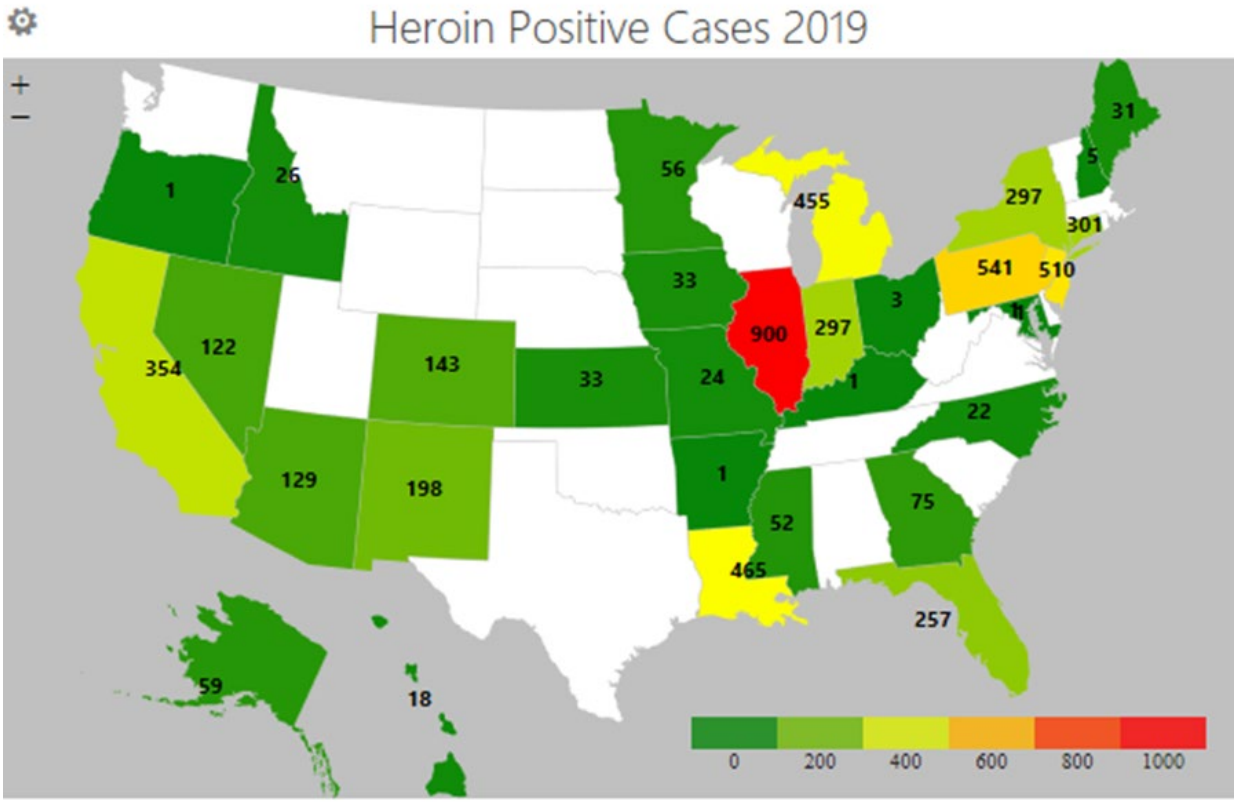

Figure 6. 2019 Geographical Distribution of Heroin Positive Cases

Upon further review of the data, an interesting trend was observed related to commonly used precursor chemicals or byproducts, including 4-ANPP and acetylfentanyl (Figure 7). Beginning in mid-May 2019, there was a significant uptick in the number of 4-ANPP positive results. Simultaneously, the detection of acetylfentanyl began to drop and was reduced by 50%

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

by the end of the year. Both of these shifts occurred while the number of fentanyl positive results remained relatively stable. Based on these trends, it can be hypothesized that the primary route of synthesis may have changed.

Following the Janssen route of synthesis, fentanyl is synthesized through the intermediate benzylfentanyl. Conversely, the Siegfried route creates 4-ANPP and uses this intermediate to produce fentanyl. Addition of the propionyl group to fentanyl is also different for each synthetic pathway: the Janssen route uses propanoic anhydride and the Siegfried route uses propionyl chloride. Based on this chemistry, it is hypothesized that the production of fentanyl switched from the Janssen route (as reported by the DEA) to the Siegfried route, which could explain the increase in the detection of 4-ANPP as leftover by-product.

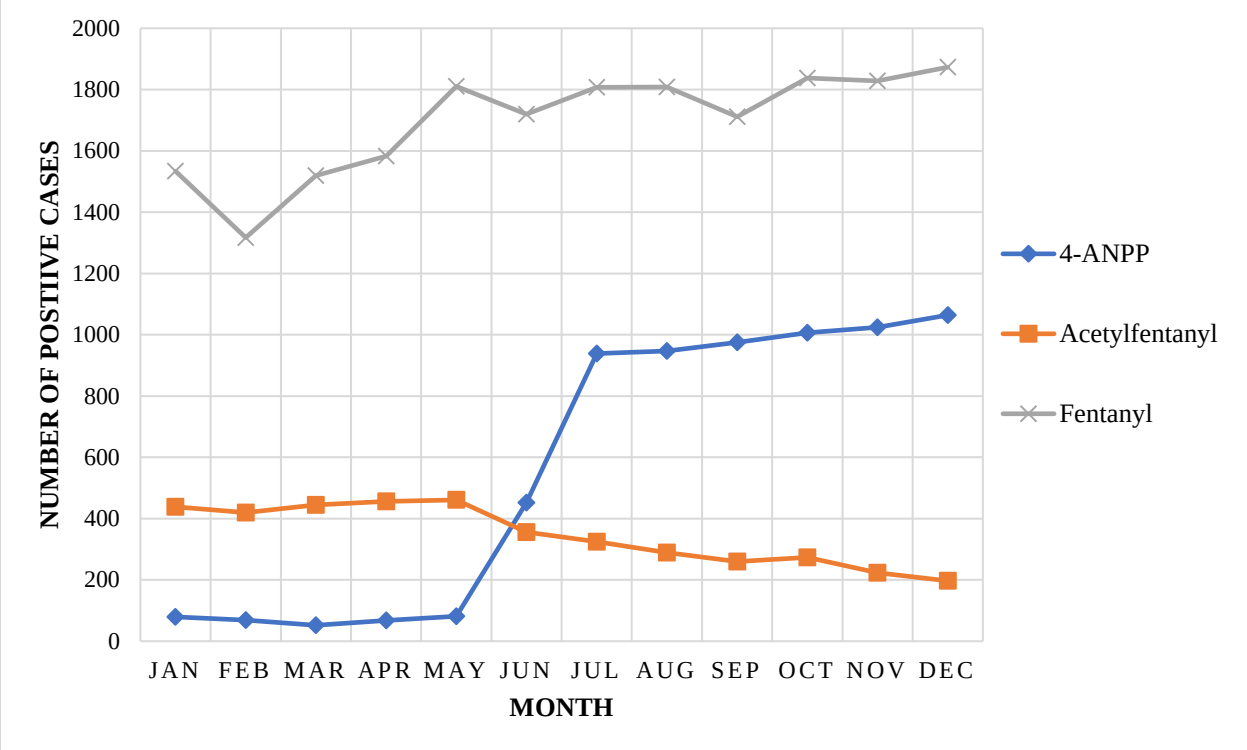

Figure 7. 2019 Positivity for Fentanyl, 4-ANPP and Acetylfentanyl by Month

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

3.2 Novel Opioids

Data related to the confirmation of novel opioids in blood was collected between the second half of 2018 (June – December) and all of 2019 (Figure 8). The total number of novel opioid cases in all of 2019 (774) was less than the total number of cases in just the last six months of 2018. Para-fluoroisobutyrylfentanyl (FIBF)/para-fluorobutyrylfentanyl showed a significant decline in positivity over the 18-month period, with 106 positive cases in June 2018 which decreased to just three positive cases in December 2019. Also, in June of 2018, cyclopropylfentanyl and methoxyacetylfentanyl peaked in positivity with 25 and 17 reported cases, respectively, which decreased to just one case for cyclopropylfentanyl and two cases for methoxyacetylfentanyl reported in December 2019. Other novel opioids, such as valerylfentanyl and carfentanil have maintained some persistence in positivity with upticks followed by sharp declines.

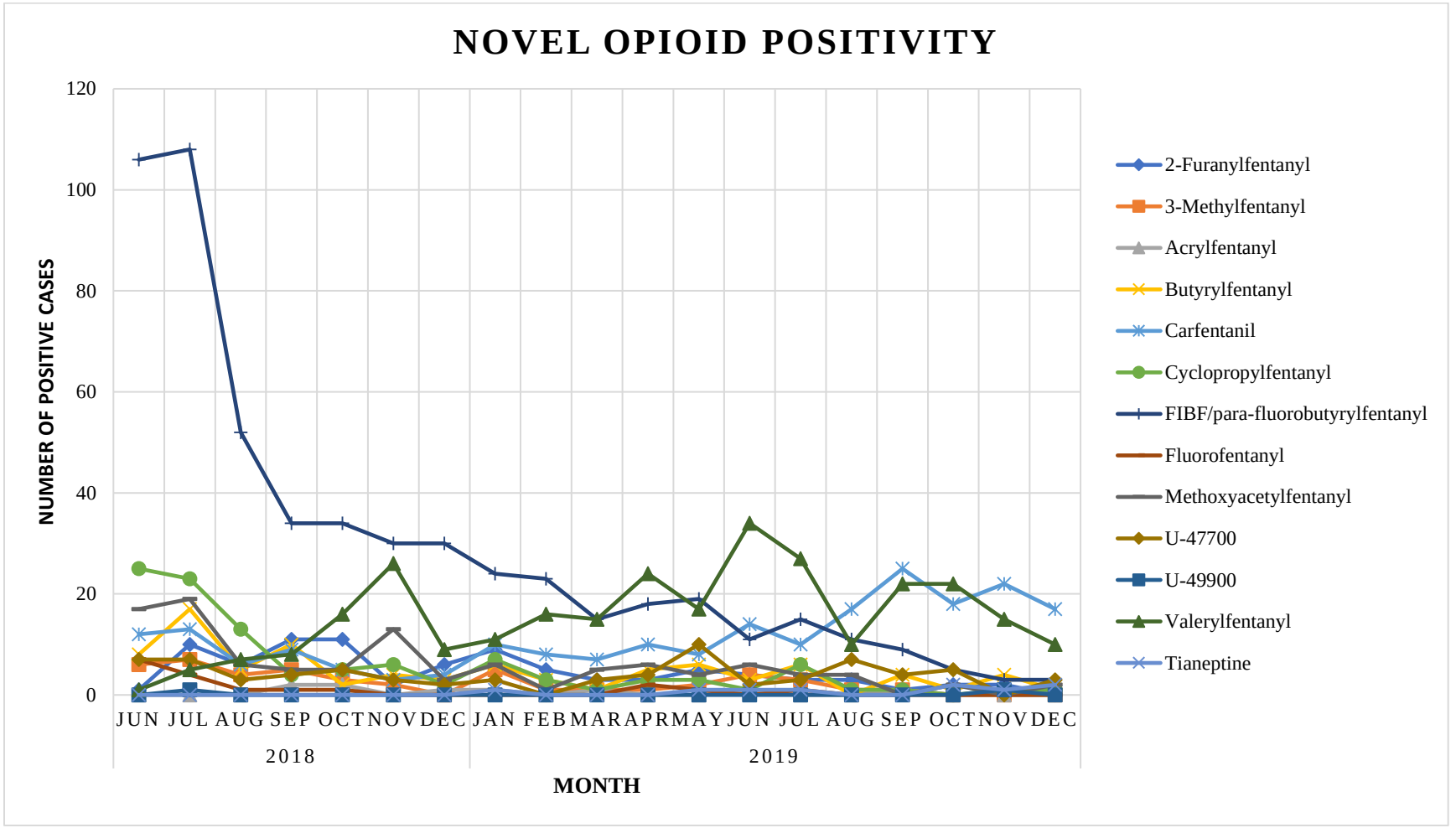

Figure 8. Novel Opioid Positivity

2018

In the six months of data for 2018, there were a total of 851 cases positive for one or more novel opioid. Para-fluoroisobutyrylfentanyl (p-FIBF) accounted for the highest positivity with 384 cases followed by cyclopropylfentanyl with 78 cases, valerylfentanyl with 72 cases, and methoxyacetylfentanyl with 68 cases.

With respect to the most commonly encountered novel opioids (p-FIBF, cyclopropylfentanyl, valerylfentanyl, and methoxyacetylfentanyl), demographic information and the type of agency submitting the case were also tabulated. For p-FIBF, there were 384 positive cases that included 246 (64%) males, 100 (26%) females, and 38 (10%) individuals with an unknown sex. The mean and median age were 39 (±11) and 38, respectively, with an age range of 19-68. Eighty-eight (88%) percent of the cases submitted came from death investigators with 5% coming from law enforcement agencies, 5% from hospitals, and 2% from other agencies.

Cyclopropylfentanyl was confirmed in 77 cases, 41 (53%) of which were male, 15 (19%) female, and 22 (28%) with an unknown sex. The mean and median age were 35 (±10) and 38, respectively, with an age range of 19-62. The majority (90%) of cases were submitted by death investigators, followed by 9% submitted by a law enforcement agency with the remaining 1% coming from an attorney.

In the valerylfentanyl positive cases (n=72), 49 (68%) were males, 15 (21%) were females, and 8 (11%) were unknown. The mean and median ages were 39 (±13) and 38, with an age range of 20-69. Ninety (90%) of the valerylfentanyl cases were submitted by death investigators, 4% by hospitals, and 3% each by law enforcement agencies and reference

laboratories. The 6-month geographic distribution of the novel opioid cases in the United States for 2018 is shown in the figure below (Figure 9).

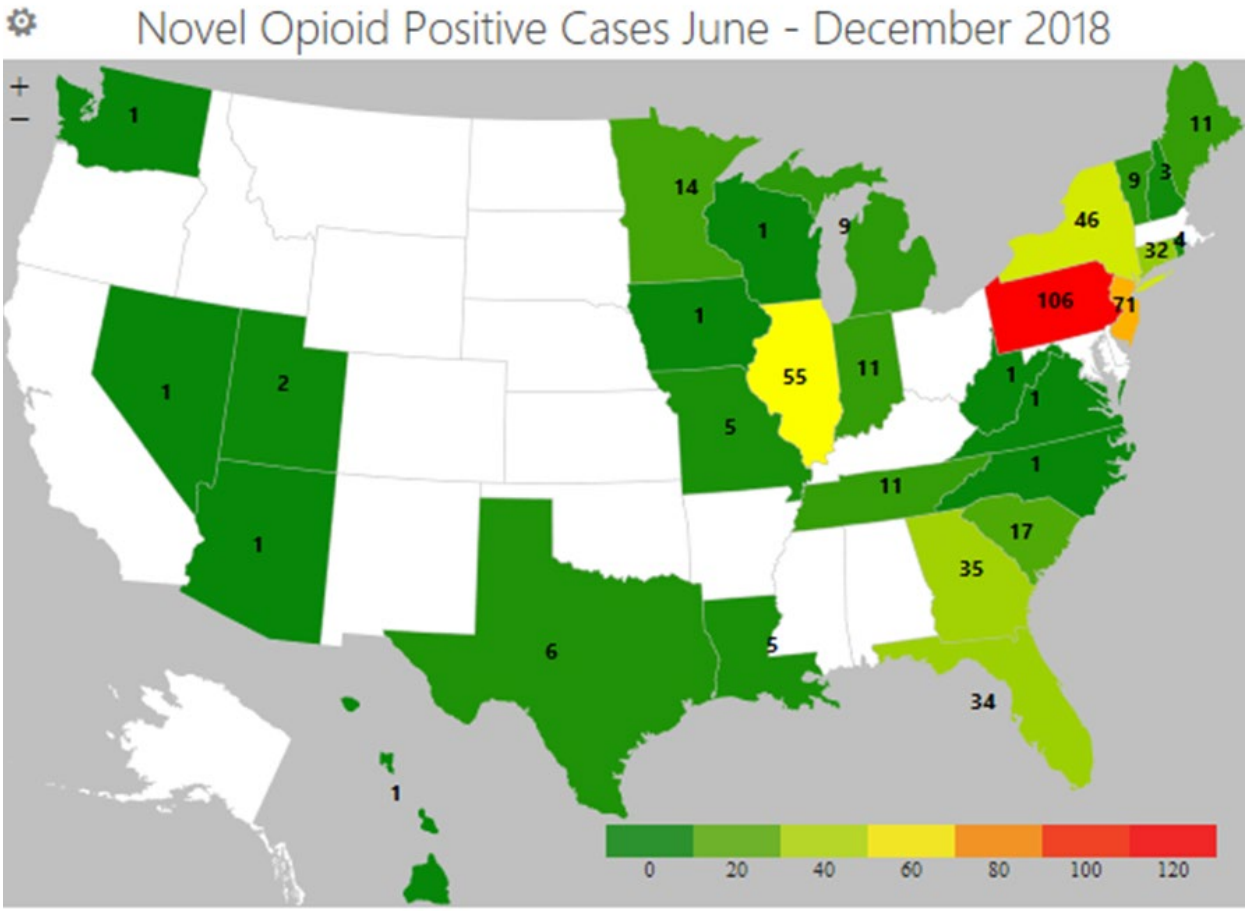

Figure 9. 2018 Geographical Distribution of Novel Opioid Positive Cases

2019

In 2019, the total number of reported cases for novel opioids decreased from cases which were reported during that last six months of 2018. The number of cases in all of 2019 (774) was compared to the number reported only in six months of 2018 (851). There was a change in the reporting of p-FIBF, where previously it was reported as a unique analyte; however, for all of the 2019 data it was reported as FIBF/para-fluorobutyrylfentanyl due to the isomers not being chromatographically separated. Valerylfentanyl accounted for the highest positivity of the year

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

with 223 confirmed cases followed by carfentanil with 166 confirmed cases and FIBF/para-fluorobutyrylfentanyl with 156 positive cases.

With respect the demographics associated with the valerylfentanyl cases, of the 223 positive cases, 140 (63%) were males, 51 (23%) were females, and 32 (14%) cases had an unknown sex. The mean and median age was 39 (±11) and 44, respectively with an age range of 18-77 years old. Ninety-four percent (94%) of the cases were submitted by death investigators, 3% submitted by both law enforcement, 2% by universities, and the remaining 1% from other agencies. For the carfentanil demographics (n=165), they were as follows: mean age 37 (±14), median 44, range 3-74, 109 males (66%), 43 females (26%), and 14 (8%) cases with an unknown sex. With respect to the submitting agency, 73% came from death investigators, 21% from law enforcement, 5% from hospitals, and 1% from other agencies. The geographic distribution of novel opioids in the United States for 2019 can be found in Figure 10.

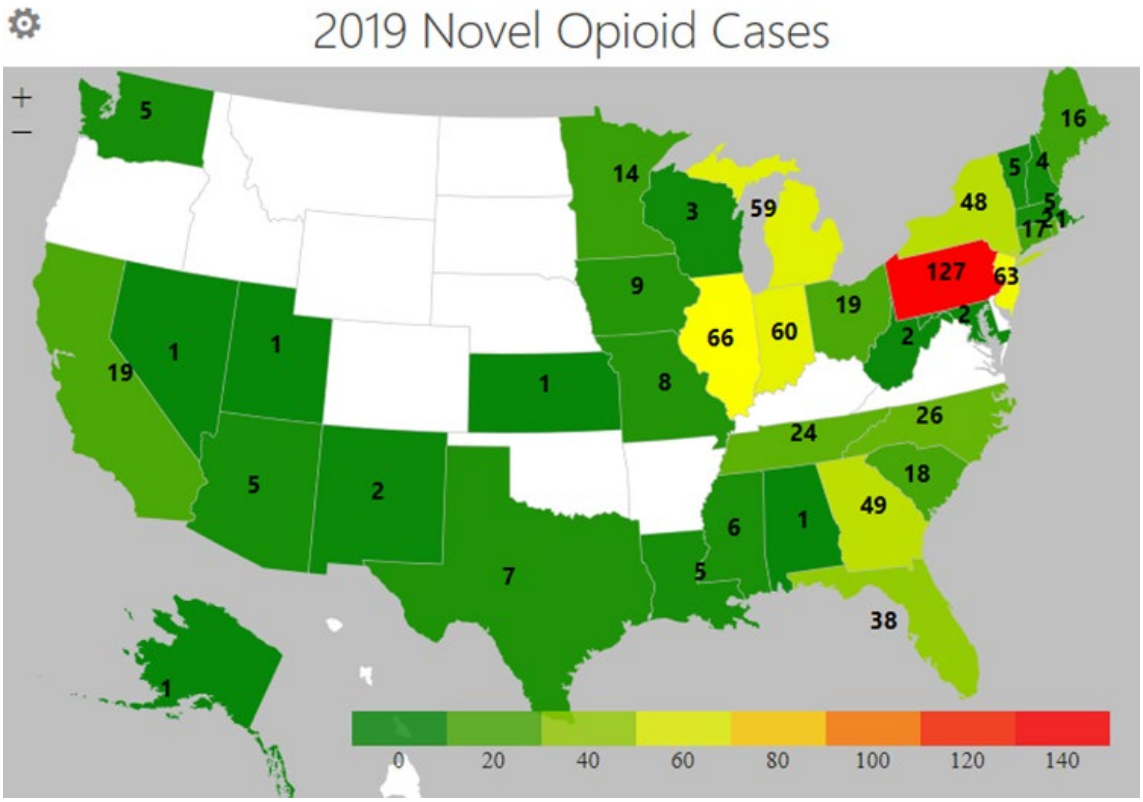

Figure 10. 2019 Geographical Distribution of Novel Opioid Positive Cases

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

3.3 Data-mining

Between January and December 2018, twelve new opioids and fentanyl analogs were identified that were not included in the original scope of testing (Table 7). Between January and December 2019, seven new opioids and fentanyl analogs were identified that were not included in the original scope of testing (Table 8).

Table 7. 2018 Data-mining Results

Analyte Name	# Identified	Month of 1st Detection
Isopropyl U-47700	10	March 2018
Benzylfentanyl*	9	January 2018
Benzylfuranylfentanyl*	9	May 2018
Phenylfentanyl	4	January 2018
3,4-Methylenedioxy U-47700	3	January 2018
Alpha'-Hydroxyacetylfentanyl	2	August 2018
Alpha-Methylbutyrylfentanyl	2	June 2018
N-Methylnorfentanyl*	2	September 2018
ortho/meta/para-Fluorofuranylfentanyl	2	December 2018
Phenylbenzylfentanyl*	2	February 2018
4'/*para*-Methylfentanyl	1	April 2018
Despropionyl-*ortho*/3-Methylfentanyl*	1	August 2018

*Precursor material

Table 8. 2019 Data-mining Results

Analyte Name	# Identified	Month of 1st Detection
Isotonitazene	60	July 2019
Ortho/Meta/Para-fluorofuranylfentanyl	8	December 2018
Piperidylthiambutene	9	June 2019
2-Methyl AP-237	4	July 2019
Benzylfuranylfentanyl*	3	May 2018
3,4-Difluoro U-47700	2	November 2019
4-Phenyl U-51754	1	November 2019

*Precursor material

Isopropyl U-47700 was first identified in a biological fluid in May 2018 and added to the emerging database in August of 2018. Through retrospective data-mining, there were seven cases that presumptively screened positive for Isopropyl U-47700, two of which were prior to

May 2018 when the first identification Isopropyl U-47700 in the United States was made (35) (Figure 11).

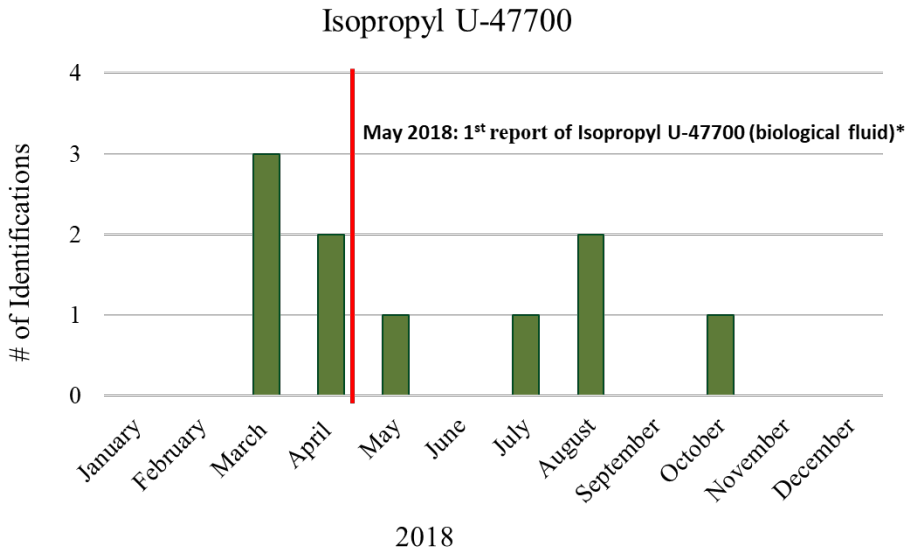

Figure 11. Data-mining Results for Isopropyl U-47700

Another example of retrospectively identifying an analyte in a casework was seen with isotonitzaene. Isotonitazene was first identified as an emerging opioid in August 2019, where it was detected in a seized drug case in Europe and in toxicology casework in Canada (36). In August 2019, the standard was added to the database and identified in six cases that month (Figure 12). In reprocessing previously acquired data, isotonitazene was found in two cases in July 2019. Continuing to process the existing data, there were over 60 screen positive cases that were identified in 2019, all of which were identified without the need for retesting the sample. Due to the significant number of positive cases that were identified via data-mining, a public health alert was issued in November 2019 to warn forensic laboratories about the increased incidence of isotonitazene positive cases (37).

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

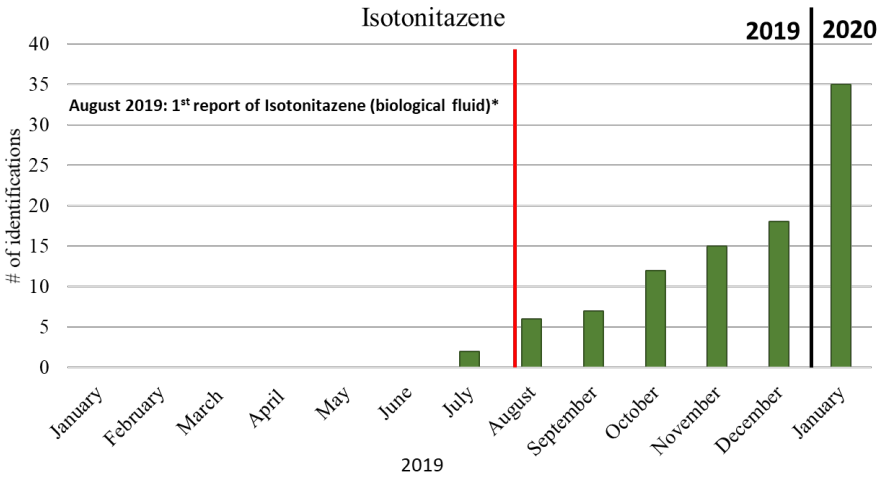

Figure 12. Data-mining Results for Isotonitazene

3.4 Toxic Adulterants

2019 cases that confirmed positive for a legacy opioid or novel opioid were examined for the presence of any of the following toxic adulterants: diltiazem, diphenhydramine, levamisole, and xylazine. When excluding samples that were positive for multiple legacy opioids and/or novel opioids due to concurrent use, in 2019 there were a total of 4,542 cases that confirmed positive for a toxic adulterant. Diphenhydramine was confirmed positive in 3,076 cases followed by xylazine in 779 cases, levamisole in 506, and diltiazem in 181 cases. Reported in Table 9 is the percent positivity for a toxic adulterant by analyte.

Table 9. Toxic Adulterant Positivity by Analyte

Analyte	Count of Analyte	Percent Levamisole	Percent Diltiazem	Percent Diphenhydramine	Percent Xylazine
2-Furanylfentanyl	47	2.1	0.0	21.3	6.4
3-Methylfentanyl	22	0.0	0.0	4.5	13.6
4-ANPP	6756	2.5	0.3	12.5	7.4
Acetylfentanyl	4145	2.4	0.1	11.8	3.7
Acrylfentanyl	11	0.0	0.0	9.1	0.0
Butyryl/Isobutyrylfentanyl	47	2.1	0.0	21.3	2.1
Carfentanil	173	1.2	0.0	15.0	4.6
Codeine	1507	0.5	0.9	8.2	0.7
Cyclopropylfentanyl	35	2.9	0.0	20.0	0.0

Dihydrocodeine / Hydrocodol	2488	0.6	1.3	11.3	0.2
Fentanyl	20342	2.1	0.2	8.2	3.8
FIBF/para-fluorobutyrylfentanyl	163	4.9	0.0	5.5	2.5
Fluorofentanyl	11	0.0	0.0	0.0	0.0
Heroin	6544	2.0	0.2	11.4	3.4
Hydrocodone	4765	0.5	0.9	8.8	0.3
Hydromorphone	2844	0.4	0.9	10.5	0.5
Methadone	2902	0.9	0.2	9.4	1.1
Methoxyacetylfentanyl	46	2.2	2.2	8.7	0.0
Morphine	6832	0.8	0.5	7.5	0.9
Oxycodone	5936	0.6	1.0	8.2	0.4
Oxymorphone	3337	0.3	1.2	9.1	0.5
Tetrahydrofuranfentanyl	6	0.0	0.0	0.0	0.0
Tianeptine	9	0.0	0.0	0.0	0.0
Tramadol	2004	0.9	1.4	14.2	0.9
U-47700	51	0.0	0.0	11.8	2.0
U-49900	8	0.0	0.0	12.5	0.0
Valerylfentanyl	230	0.9	0.4	12.6	2.6

The data was further evaluated by looking specifically at heroin positive, fentanyl positive, heroin and fentanyl positive, and novel opioid positive cases found with toxic adulterant combinations. The data can be found in figures 13-16. With respect to combinations of toxic adulterants, diphenhydramine and xylazine were most commonly found in combination in these cases, but confirmed positive to a lesser extent than when the toxic adulterant was found alone. The detection of toxic adulterants was seen far less frequently in cases where a novel opioid was detected. In novel opioid positive cases, diphenhydramine was the only toxic adulterant found in 39 cases, followed by levamisole in 7 cases. Toxic adulterant combinations were rarely found in novel opioid positive cases.

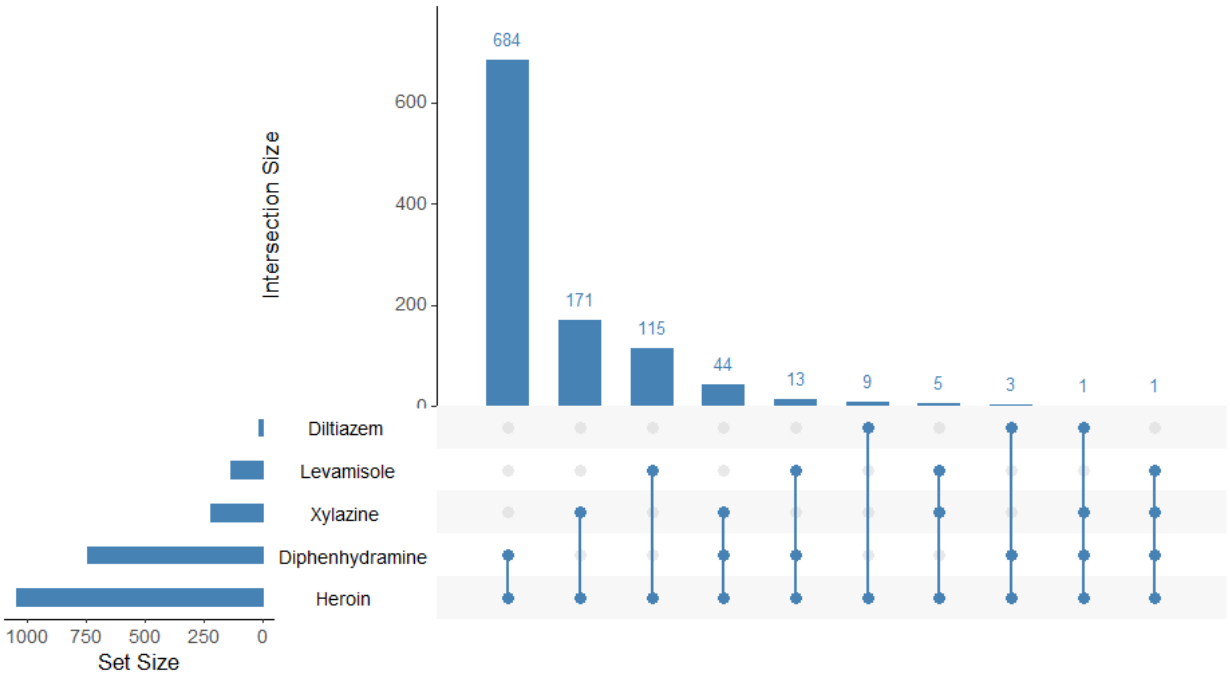

Figure 13. Toxic Adulterants Found in Heroin Positive Cases

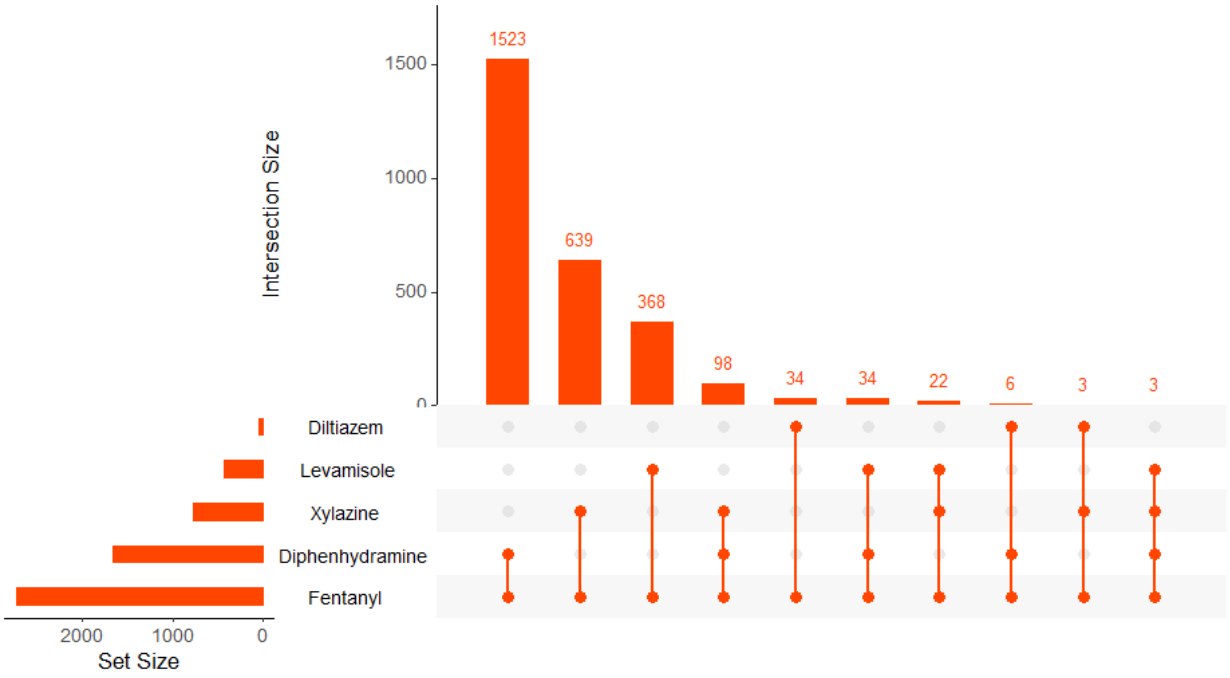

Figure 14. Toxic Adulterants Found in Fentanyl Positive Cases

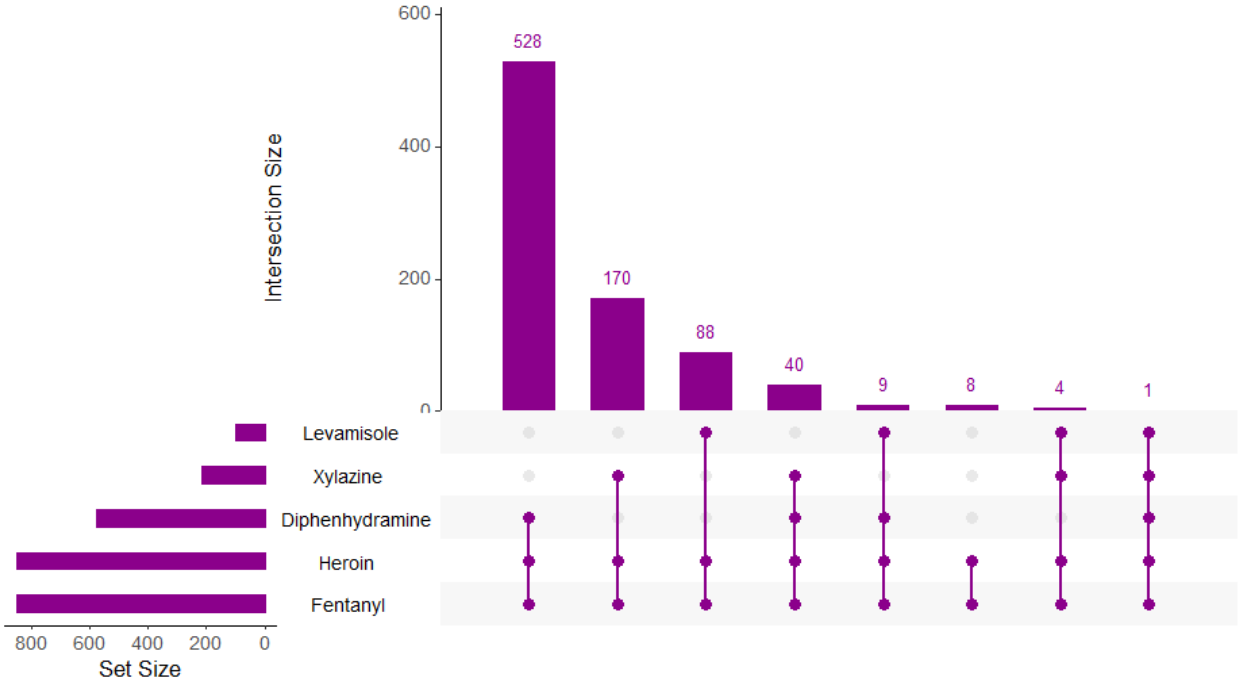

Figure 15. Toxic Adulterants Found in Heroin and Fentanyl Positive Cases

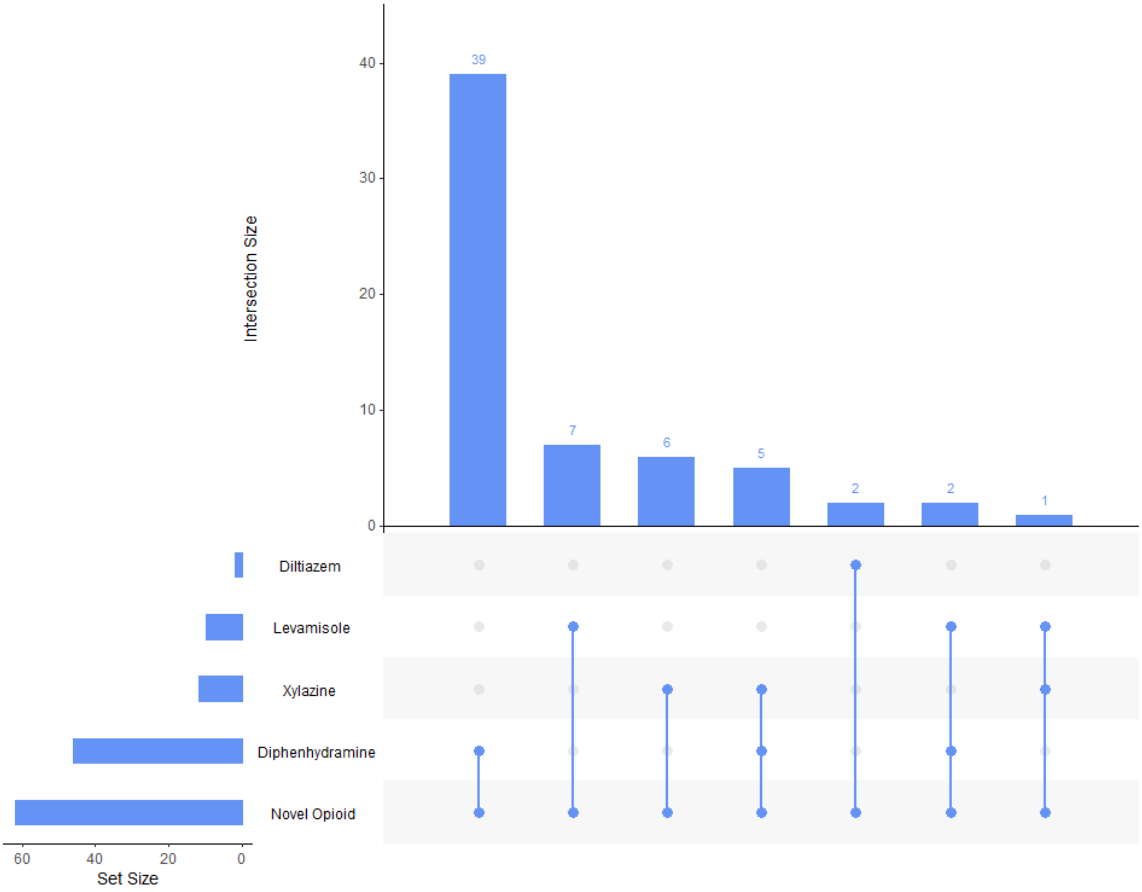

Figure 16. Toxic Adulterants Found in Novel Opioid Positive Cases

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

The caveat to the data is that many of the substances known to be toxic adulterants are also available for legitimate use. Diphenhydramine is available as an over-the-counter decongestant/sleep aid, and diltiazem is a pharmaceutical that can be administered by physicians as a part of routine care. Levamisole and xylazine are however not approved in the United States for therapeutic use. Levamisole is also a commonly found adulterant in cocaine samples; however, cocaine positivity was not included in this analysis. Both of these drugs were detected in these cases representing likely exposure through illicit drug supply.

4. Conclusions

The opioid epidemic has created a public health problem that continues to pose significant challenges to the forensic science community. Since the start of the opioid epidemic, which began with the appearance of previously synthesized opioid analgesics derived from pharmaceutical patents, the ability to identify and associate these substances with forensic cases was complicated by the frequency with which they were appearing, the constant evolution of new isomers, and the inability of laboratories to keep pace with the changing illicit market. To address these concerns, the objectives of this project were to provide insight into the opioid epidemic in real time, to provide context about the implication of newly identified analytes in forensic casework that were not known about at the time of original testing and provide a baseline for forensic toxicology pharmacoepidemiology in the area of opioids.

Through this project, we developed analytical approaches, systematic strategies, software tools, and operational workflows to create a real-time monitoring and early warning system for opioid trends in the United States that was widely disseminated within the forensic science and criminal justice communities to thousands of public health and public safety partners. NPS

Discovery (www.npsdiscovery.org), an interactive website, was developed as a resource that warehouses information and reports on emerging analytes for further dissemination of this information to stakeholders and affected communities. To our knowledge, there is no other comprehensive reporting system that represents data at the national level. Current reporting systems typically have data from targeted areas or lack real-time reporting.

Within the data presented in this report, we have shown over the course of 18 months and through the analysis of over 100,000 samples, fentanyl positivity has continued to increase throughout 2019, heroin has remained stable, and novel opioids have significantly dropped in overall positivity. We postulate the market will see sustained fentanyl positivity and migrate toward drug combinations containing fentanyl with isolated pulses in the appearance of emerging opioids. To that end, it is imperative that we retain a real-time monitoring system that can provide laboratories with tangible evidence about what target analytes to include in their scope of analysis. Surveillance measures such as the model we have developed and described herein are critical to reducing opioid-involved deaths.

In addition to providing a real-time monitoring system, we have demonstrated there is a short lag time between new identifications in seized drug cases and detecting these substances in toxicological cases. Through the use of data-mining, we have shown that new analytes are often identified in cases several months prior to formally identifying a new substance. The value of data-mining is that these new identifications can be made by reprocessing the existing raw data against an updated database without the need for retesting the sample, which saves time and resources.

Data-mining also allows laboratories the opportunity to investigate the relative prevalence of emerging analytes and evaluate whether or not the laboratory should move forward

with method development and validation, or if the more economical approach would be to outsource the confirmation testing. These findings reinforce the value of laboratories frequently updating their scope of testing, and continuing to investigate cases that appear to be an opioid-related death without a significant toxicological opioid-related finding. In such cases, laboratories could implement a targeted query of the existing data as emerging opioids are identified.

The data collected as part of this project shows that the opioid epidemic is far from over. There is no indication that opioid positive cases are declining, and continued resources are needed for monitoring the trajectory opioid prevalence in the United States. Generating real-time data is a critical component to justifying the need for additional funding to remain current with opioid trends within the forensic science community and provide information related to this public health crisis.

5. Acknowledgements

The authors of this report would like to acknowledge Sherri Kacinko, Donna Papsun, and Cristina Carrico at NMS Labs for their involvement in this project, Melissa Fogarty and Alex Krotulski at the Center for Forensic Science Research and Education, and David Buzby at the New Jersey State Police Office of Forensic Sciences. The authors would also like to recognize our key collaborator on this project, NMS Labs.

References

1. Rudd, R.A., Aleshire, N., Zibbell, J.E. and Matthew Gladden, R. (2016) Increases in Drug and Opioid Overdose Deaths-United States, 2000-2014. *American Journal of Transplantation*, **16**, 1323–1327.

2. Warner, M., Bastian, B., Hedegaard, H. and Trinidad, J. (2016) Drugs Most Frequently Involved in Drug Overdose Deaths: United States, 2010–2014, National Vital Statistics Reports Volume 65, Number 10 December 20, 2016. 2016.

3. Rudd, R.A., Seth, P., David, F. and Scholl, L. (2016) Increases in Drug and Opioid-Involved Overdose Deaths — United States, 2010–2015. *MMWR. Morbidity and Mortality Weekly Report*, **65**, 1445–1452.

4. Spencer, M., Warner, M., Bastian, B., Trinidad, J. and Hedegaard, H. (2019) Drug Overdose Deaths Involving Fentanyl, 2011-2016, National Vital Statistics Reports Volume 68, Number 3 March 21, 2019. **68**, 19.

5. Hedegaard, H., Miniño, A. and Warner, M. (2020) Drug Overdose Deaths in the United States, 1999–2018. 2020.

6. Wilson, N., Kariisa, M., Seth, P., Iv, H.S. and Davis, N.L. (2020) Drug and Opioid-Involved Overdose Deaths – United States, 2017–2018. **69**, 8.

7. Jannetto, P.J., Helander, A., Garg, U., Janis, G.C., Goldberger, B. and Ketha, H. (2019) The Fentanyl Epidemic and Evolution of Fentanyl Analogs in the United States and the European Union. *Clinical Chemistry*, **65**, 242–253.

8. Bowen, D.A., O'Donnell, J. and Sumner, S.A. (2019) Increases in Online Posts About Synthetic Opioids Preceding Increases in Synthetic Opioid Death Rates: a Retrospective Observational Study. *Journal of General Internal Medicine*, **34**, 2702–2704.

9. Morrow, J.B., Ropero-Miller, J.D., Catlin, M.L., Winokur, A.D., Cadwallader, A.B., Staymates, J.L., et al. (2019) The Opioid Epidemic: Moving Toward an Integrated, Holistic Analytical Response. *Journal of Analytical Toxicology*, **43**, 1–9.

10. Ruangyuttikarn, W., Law, M.Y., Rollins, D.E. and Moody, D.E. (1990) Detection of Fentanyl and its Analogs by Enzyme-Linked Immunosorbent Assay*. *Journal of Analytical Toxicology*, **14**, 160–164.

11. Warrington, J.S., Walsh, A., Baker, E., Lozier, D. and Belec, A. (2018) Keeping Up with Fentanyl: Failure to Do So Is Not an Option. *The Journal of Applied Laboratory Medicine*, **3**, 148–151.

12. Zacca, J.J., Botelho, É.D., Vieira, M.L., Almeida, F.L.A., Ferreira, L.S. and Maldaner, A.O. (2014) Brazilian Federal Police drug chemical profiling — The PeQui Project. *Science & Justice*, **54**, 300–306.

13. Maldaner, A.O., Botelho, É.D., Zacca, J.J., Camargo, M.A., Braga, J.W. and Grobério, T.S. (2015) Brazilian Federal District Cocaine Chemical Profiling - Mass Balance Approach and New Adulterant Routinely Quantified (Aminopyrine). *Journal of the Brazilian Chemical Society*, 2015: 10.5935/0103-5053.20150088. http://www.gnresearch.org/doi/10.5935/0103-5053.20150088 (9 March 2020).

14. Bernardo, N.P., Siqueira, M.E.P.B., de Paiva, M.J.N. and Maia, P.P. (2003) Caffeine and other adulterants in seizures of street cocaine in Brazil. *International Journal of Drug Policy*, **14**, 331–334.

15. Botelho, É.D., Cunha, R.B., Campos, A.F.C. and Maldaner, A.O. (2014) Chemical Profiling of Cocaine Seized by Brazilian Federal Police in 2009-2012: Major Components. *Journal of the Brazilian Chemical Society*, 2014: 10.5935/0103-5053.20140008. http://www.gnresearch.org/doi/10.5935/0103-5053.20140008 (9 March 2020).

16. de Souza, L.M., Rodrigues, R.R.T., Santos, H., Costa, H.B., Merlo, B.B., Filgueiras, P.R., et al. (2016) A survey of adulterants used to cut cocaine in samples seized in the Espírito Santo State by GC–MS allied to chemometric tools. *Science & Justice*, **56**, 73–79.

17. Floriani, G., Gasparetto, J.C., Pontarolo, R. and Gonçalves, A.G. (2014) Development and validation of an HPLC-DAD method for simultaneous determination of cocaine, benzoic acid, benzoylecgonine and the main adulterants found in products based on cocaine. *Forensic Science International*, **235**, 32–39.

18. Fukushima, A.R., Carvalho, V.M., Carvalho, D.G., Diaz, E., Bustillos, J.O.W.V., Spinosa, H. de S., et al. (2014) Purity and adulterant analysis of crack seizures in Brazil. *Forensic Science International*, **243**, 95–98.

19. Grobério, T.S., Zacca, J.J., Botelho, É.D., Talhavini, M. and Braga, J.W.B. (2015) Discrimination and quantification of cocaine and adulterants in seized drug samples by infrared spectroscopy and PLSR. *Forensic Science International*, **257**, 297–306.

20. Lapachinske, S.F., Okai, G.G., dos Santos, A., de Bairros, A.V. and Yonamine, M. (2015) Analysis of cocaine and its adulterants in drugs for international trafficking seized by the Brazilian Federal Police. *Forensic Science International*, **247**, 48–53.

21. Magalhães, E.J., Nascentes, C.C., Pereira, L.S.A., Guedes, M.L.O., Lordeiro, R.A., Auler, L.M.L.A., et al. (2013) Evaluation of the composition of street cocaine seized in two regions of Brazil. *Science & Justice*, **53**, 425–432.

22. Marcelo, M.C.A., Mariotti, K.C., Ferrão, M.F. and Ortiz, R.S. (2015) Profiling cocaine by ATR–FTIR. *Forensic Science International*, **246**, 65–71.

23. Penido, C.A.F.O., Pacheco, M.T.T., Zângaro, R.A. and Silveira, L. (2015) Identification of Different Forms of Cocaine and Substances Used in Adulteration Using Near-infrared Raman Spectroscopy and Infrared Absorption Spectroscopy. *Journal of Forensic Sciences*, **60**, 171–178.

24. Gaertner, J., Radbruch, L., Giesecke, T., Gerbershagen, H., Petzke, F., Ostgathe, C., et al. (2006) Assessing cognition and psychomotor function under long-term treatment with controlled release oxycodone in non-cancer pain patients. *Acta Anaesthesiologica Scandinavica*, **50**, 664–672.

25. Knowles, L., Buxton, J.A., Skuridina, N., Achebe, I., LeGatt, D., Fan, S., et al. (2009) Levamisole tainted cocaine causing severe neutropenia in Alberta and British Columbia. *Harm Reduction Journal*, **6**, 30.

26. Czuchlewski, D.R., Brackney, M., Ewers, C., Manna, J., Fekrazad, M.H., Martinez, A., et al. (2010) Clinicopathologic Features of Agranulocytosis in the Setting of Levamisole-Tainted Cocaine. *American Journal of Clinical Pathology*, **133**, 466–472.

27. Hunter, L., Gordge, L., Dargan, P.I. and Wood, D.M. (2011) Methaemoglobinaemia associated with the use of cocaine and volatile nitrites as recreational drugs: a review: Recreational drug-related methaemoglobinaemia. *British Journal of Clinical Pharmacology*, **72**, 18–26.

28. Chakladar, A., Willers, J.W., Pereskokova, E., Beaumont, P.O. and Uncles, D.R. (2010) White powder, blue patient: Methaemoglobinaemia associated with benzocaine-adulterated cocaine. *Resuscitation*, **81**, 138–139.

29. Veronese, F.V., Dode, R.S.O., Friderichs, M., Thomé, G.G., Silva, D.R. da, Schaefer, P.G., et al. (2016) Cocaine/levamisole-induced systemic vasculitis with retiform purpura and pauci-immune glomerulonephritis. *Brazilian Journal of Medical and Biological Research*, **49**, e5244.

30. Muirhead, T.T. and Eide, M.J. (2011) Toxic Effects of Levamisole in a Cocaine User. *New England Journal of Medicine*, **364**, e52.

31. Auffenberg, C., Rosenthal, L.J. and Dresner, N. (2013) Levamisole: A Common Cocaine Adulterant with Life-Threatening Side Effects. *Psychosomatics*, **54**, 590–593.

32. Belfonte, C.D., Shanmugam, V.K., Kieffer, N., Coker, S., Boucree, S. and Kerr, G. (2013) Levamisole-induced occlusive necrotising vasculitis in cocaine abusers: an unusual cause of skin necrosis and neutropenia. *International Wound Journal*, **10**, 590–596.

33. Sari, A. (2019) Nephrotoxic Effects of Drugs. *Poisoning in the Modern World - New Tricks for an Old Dog?*, January 24, 2019: 10.5772/intechopen.83644. https://www.intechopen.com/books/poisoning-in-the-modern-world-new-tricks-for-an-old-dog-/nephrotoxic-effects-of-drugs (10 March 2020).

34. Brunt, T. (2012) Monitoring illicit psychostimulants and related health issues. BOXPress ; Universiteit van Amsterdam [Host, Oisterwijk; Amsterdam.

35. Krotulski, A. and Logan, B. (2018) Isopropyl-U-47700 Toxicology Analytical Report. *NPS Discovery - Monographs*, 2018. https://www.npsdiscovery.org/wp-content/uploads/2019/06/Isopropyl-U-47700_051818_ToxicologyAnalyticalReport.pdf (20 March 2020).

36. Brooks-Lim, E. and Chatterton, C. (2019) Novel Psychoactive Substances (NPS) Detection in Alberta Casework (August 2019 update). 2019.

37. Krotulski, A., Papsun, D., Fogarty, M., Nelson, L. and Logan, B. (2019) Public Alert Isotonitazene NPS-Discovery. November 2019. https://www.npsdiscovery.org/wp-content/uploads/2019/11/Public-Alert_Isotonitazene_NPS-Discovery_111919-1.pdf.