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Assessment of the Contribution to Drug Impaired Driving from Emerging and Undertested Drugs

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Summary of the Project

Impaired driving has become a problem and a public health concern internationally and within the United States (US). The use of illicit substances, prescription medications and/or over-the-counter medications has continued to rise over the last decade and in turn resulted in the potential for more drivers to be on the road while using any of the substances mentioned. According to the results of the 2013-2014 National Roadside Survey of Alcohol and Drug Use by Drivers, approximately 22% of randomly stopped drivers tested positive for drugs in oral fluid or blood specimens (1). While the simple detection of a drug in these matrices does not imply impairment, illegal substances without medicinal use were detected in up to 15% of drivers during the nighttime hours of the weekend. More recent data collected found that 55.8% of the injured or killed roadway users tested positive for one or more drugs (including alcohol) (2). Increasingly, drug impaired driving is becoming a focus in the US for law enforcement and traffic safety agencies, and applying a comprehensive and systematic approach to detection, investigation, and analysis is key to successful prosecutions (3, 4).

Many different types of laboratories perform drug testing in support of law enforcement DUID cases, Drug Recognition Expert (DRE) programs, and investigations of traffic fatalities. These include private commercial clinical and forensic laboratories, government (state, local and municipal) laboratories, and hospital and clinical laboratories. Each may have a different focus, resources, and levels of expertise available with respect to planning, executing and reporting results of this testing. Beginning in 2004, the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID) (previously the Committee of Alcohol and Other Drugs (CAOD)), started an initiative to standardize toxicology laboratory testing practices for cases involving DUID by surveying the testing scope and analytical cutoffs being used for blood and urine drug testing by those laboratories (5). Since the first publication in 2007, a total of three iterations of recommendations for toxicological investigation of drug impaired driving have been published based on the input from laboratories surveyed across the US (6–8). The 2017 recommendations were most recently cited by the American Standards Board (ASB) as the basis for their standards for forensic toxicology testing in impaired driving investigations (9).

As part of these recommendations, drug groups have been divided into two groups: Tier I and Tier II (Table 1 and 2). Tier I drugs encompass the most frequently encountered drugs in DUID casework and those that can be analytically detected by immunoassay and confirmed using gas chromatography/mass spectrometry (GC/MS). As such, these drugs are included in the mandatory scope of testing for all impaired driving and traffic fatality cases. Tier II compounds are drugs that were classified as optional for laboratories to include in the scope of their testing because their prevalence was believed to be lower, or the means of detection were not widely available to all kinds of testing laboratories. In addition, Tier II includes drugs that may have regional significance, but could not be justified in a national recommendation. Tier II therefore represents a useful staging or assessment designation for the curation and updating of the Tier I panel. The predicament is that there is less testing performed of Tier II compounds to properly assess their prevalence and therefore their contribution to DUID arrests or fatalities.

Table 1. Recommended Scope for Tier I Testing

DRE category; cannabis	***DRE category; CNS depressants ctnd.***
THC	Nordiazepam
THC-COOH	Oxazepam
11-OH-THC	Temazepam
DRE category; CNS stimulants	***DRE category; narcotic analgesics***
Methamphetamine	Codeine
Amphetamine	6-Acetylmorphine
MDMA	Buprenorphine
MDA	Norbuprenorphine
Cocaine	Fentanyl
Benzoylecgonine	Hydrocodone
Cocaethylene	Hydromorphone
DRE category; CNS depressants	Methadone
Carisoprodol	Morphine
Meprobamate	Oxycodone
Zolpidem	Oxymorphone
Alprazolam	Tramadol
Alpha-Hydroxyalprazolam	O-desmethyltramadol
Clonazepam	
7-Aminoclonazepam	
Lorazepam	
Diazepam	

Table 2. Recommended Drugs for Tier II Testing

DRE category; cannabis	***DRE category; CNS depressants ctnd.***
Synthetic cannabinoids	Phenytoin
DRE category; CNS stimulants	Pregabalin
Cathinones	Secobarbital
Methylphenidate	Topiramate
Mitragynine	Trazodone
DRE category; CNS depressants	Tricyclic antidepressants
Atypical antipsychotics	Valproic acid
Barbiturates	Zopiclone
Carbamazepine	***DRE category; narcotic analgesics***
Chlordiazepoxide	Fentanyl analogs
Chlorpheniramine	Novel opioids
Cyclobenzaprine	Tapentadol
Diphenhydramine	***DRE category; dissociative drugs***
Doxylamine	Dextromethorphan
Gabapentin	Ketamine
Gamma-hydroxybutyrate	PCP
Hydroxyzine	***DRE category; inhalants***
Lamotrigine	Inhalant class

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	Mirtazapine			Difluoroethane
	Novel benzodiazepines		***DRE category; hallucinogens***	
				Hallucinogens

Major Goals and Objectives

To better characterize the contribution to drug impaired driving in the US associated with compounds which are not included as part of the standardized scope of testing (Tier I compounds) set forth by the NSC-ADID recommendations, the goal of this research was to comprehensively test blood samples collected and submitted for analysis to a reference laboratory for both Tier I and Tier II drugs and other emergent substances, including NPS in suspected DUID cases. Deidentified samples were transferred to CFSRE for analysis using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF) for all Tier I and II drugs to evaluate the frequency to which these drugs and drug combinations are identified with in the sample population. Additional objectives included a retrospective analysis of four years of DUID drug concentration data for all Tier I analytes and several Tier II analytes to provide insight into changing patterns of use and concentrations within this population to better aid toxicologists in their interpretation of these results.

Research Questions

The main research questions aimed to be addressed through this research included validating the NSC Tier I and Tier II recommendations, assessing drug positivity in cases where alcohol met an administratively determined level reported to be a threshold where drug testing is not pursued, and finally evaluating drug concentrations over a four-year period.

Research Design, Methods, Analytical and Data Analysis Techniques

Objective 1 – Sample Acquisition and Analysis

Approximately125 deidentified samples per month were transferred from NMS Labs (Horsham, PA) to the CFSRE for testing using high resolution mass spectrometry (LC-TOF). The samples were selected at random from the pool of samples submitted for suspected impaired driving cases. In addition to the samples, the blood alcohol concentration (BAC) and quantitative results from the cannabinoids panel, which included Δ9-tetrahydrocannabinol (THC), 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) were provided.

Aim 1.1 – Analysis for Basic Drugs

Samples were extracted using a simple, single-step liquid-liquid extraction (LLE) previously published method for basic drugs (10, 11). Samples were reconstituted in 100 µL of LC initial conditions. A control containing all Tier I drugs at the recommended cutoff was run with every sample batch in additional to other quality controls as outlined in the sample analysis standard operating procedure (SOP).

All samples were screened using high resolution mass spectrometry on the SCIEX TripleTOF® 5600+ LC-QTOF system (SCIEX, Framingham, MA). The parameters of the screening method

have been previously published (10, 11). Data processing was performed using PeakView (Version 2.2) and MasterView™ (Version 1.1) software. Additional details of the method are described in the literature (10, 11). Samples were processed against a regularly updated in-house library that contained all Tier I and Tier II drugs (Table 1-2). Notable exemptions from the library for Tier II drugs included valproic acid, gamma-hydroxybutyrate (GHB), secobarbital, and inhalants.

Aim 1.2 – Analysis for Synthetic Cannabinoids
Blood samples (0.5 mL) were fortified with internal standard (50 µL of a 0.2 ng/µL), basified with TRIS HCl buffer (1.0 M, pH 10.2), and subsequently extracted into methyl tert-butyl ether (MTBE, 3 mL). Resulting sample mixtures were rotated for 15 minutes prior to centrifugation at 4600 rpm for 10 minutes. The aqueous layer was frozen using a -80 °C freezer, and the supernatant was transferred for drying at 35 °C for roughly 25 minutes. Following the dry down step, samples were reconstituted in 200 µL of initial mobile phase conditions (95:5) and subsequently analyzed via LC-QTOF.

All samples were analyzed on a Sciex (Framingham, MA) TripleTOF® 5600+ QTOF coupled with a Shimadzu (Shimadzu, Kyoto, Japan) Nexera UHPLC using a Phenomenex® Kinetex C18 analytical column. The method has a total run time of 7 minutes. Chromatographic separation was achieved using ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) at a flow rate of 0.5 mL/min. Analytes were ionized via positive electrospray ionization. Data was processed using PeakView® (Version 2.2) and MasterView™ (Version 1.1). Additional method details are described in the literature (12). The synthetic cannabinoid library has over 250 parent compounds and metabolites in it and is regularly updated as new synthetic cannabinoids are available as certified reference material.

Aim 1.3 – Analysis for Gabapentin
Due to the concern surrounding gabapentin as an emerging drug and it not being extracted well in either of the two other methods, an additional targeted screening approach was made specifically for this drug. Leveraging the high concentrations at which gabapentin is found at in biological specimens, five individual blood samples were pooled together, taking 20 µL from each sample. Next, 100 µL of blank blood was added followed by the addition of 600 µL trichloroacetic acid in deionized water. Samples were then processed using the method described in Aim 1.1. If any batch of the pooled samples was positive, samples then individually acquired to determine which sample was positive.

Objective 2 – Method Development and Validation for Identified Analytes of Interest
Following the initial screening, confirmatory methods were developed for any Tier II analyte that was detected in ten or more cases, for which there was not a confirmatory method available either at CFSRE or NMS Labs. Method development and validation were performed according to the ASB “Standard Practices for Method Validation in Forensic Toxicology” document (13).

Objective 3 – DRE Evaluations
The goal of this objective was to collect DRE face sheets submitted with suspected impaired driving cases that would be de-identified, transcribed, and provided with the de-identified sample.

Objective 4 – 4-year Assessment of DUID Drug Concentrations
Confirmatory methods are available at NMS Labs for all of the NSC Tier I drugs. To also aid with the interpretation of results, historical data related to drugs confirmed in DUID cases as well as the concentrations reported in these cases collected over the past four years were pulled and tabulated.

Objective 5 – Dissemination
Work related to the project was provided to the NIJ in the form of semiannual progress reports. Data was also disseminated via presentation at professional meetings and drafted for publication.

Expected Applicability of the Research
Drug impaired driving continues to pose both public health and public safety concerns. Based on the findings of this research, 79% percent of suspected impaired driving cases were positive for drugs, some of which also contain alcohol. The data further supports the NSC-ADID Tier I recommendations; drugs detected with the greatest frequency are captured in Tier I. As noted by the recommendations, drugs in Tier II are generally found with less frequency. However, it should be noted that some Tier II drugs (diphenhydramine, gabapentin, hydroxyzine, and NPS (8-aminoclonazolam and fluorofentanyl)) were observed to have an equivalent or greater positivity rate compared to some Tier I drugs. Polysubstance use with drugs spanning multiple categories or drugs found in combination with alcohol was common. An evaluation of drug positivity relative to various BAC thresholds identified when there was a BAC of ≥0.08 g/100mL revealed drug positivity of 19%, suggesting that laboratories employing stop limit testing are missing a significant number of drug positive cases. Limiting testing based on alcohol results precludes information of drug involvement in several cases, leading to underreporting of drug contributions to impaired driving. Laboratories can use this research to evaluate their current practices and vet them against a larger dataset.

Participants and other Collaborating Organizations

Without the help of our collaborators, the work performed under this grant would not have been possible. All laboratory-based assessments and developments were completed at the CFSRE and involved Grace Cieri, Amanda Mohr, Melissa Fogarty, and Barry Logan. Collaborating agencies included NMS labs (Horsham, PA) and SCIEX (Framingham, MA).

Changes in Approach from Original Design and Reason for Change, if applicable

The initial goal of this project was to analyze over 4,000 samples for both tier I and Tier II drugs by receiving roughly 200 samples a month. However, after starting the project, it was clear keeping up with that number was not possible due to instrument time and availability combined with data processing and additional extractions needed. In the end roughly 2,500 samples were screened for Tier I and Tier II drugs, which represents a large population suitable to meet the research goals outlined above.

Outcomes

Activities/Accomplishments

Objective 1 – Sample Acquisition and Analysis

- Analyzed over 2,500 samples using the basic and the synthetic cannabinoid methods.
- Over 1,900 samples were screened for gabapentin using a protein crash.
- Quantitative data for alcohol, THC, and THC metabolites were provided by NMS labs.

Objective 2 – Method Development and Validation for Identified Analytes of Interest

- Determined that some of the most commonly seen analytes of interest were novel benzodiazepines.
- Developed and validated a quantitative method for seven novel benzodiazepines.

Objective 3 – DRE Evaluations

- No DRE evaluations were provided by our collaborators for the data set, which did not allow for any assessment or conclusions.

Objective 4 – 4-year Assessment of DUID Drug Concentrations

- Quantitative data for Tier I drugs has been reviewed from 2017 to 2020.
- Mean, median, minimum, and maximum concentrations for drugs in Tier I were compiled.

Objective 5 – Dissemination

- Presented data at conferences including IACT, SOFT, IDTS, AAFS, and the Robert F. Borkenstein Conference on "The Effects of Drugs on Human Performance and Behavior".
- Progress reports submitted to NIJ semiannually.
- Manuscripts are currently being drafted to further disseminate the data within the peer-reviewed literature.

Results and Findings

Objective 1

A total of 2,514 samples were analyzed using the basic drug screen and synthetic cannabinoid panels. Due to the concern surrounding gabapentin as an emerging drug and it not being extracted well in either of the two other methods, an additional targeted screening approach was made specifically for this drug. This procedure is described below in Figure 1.

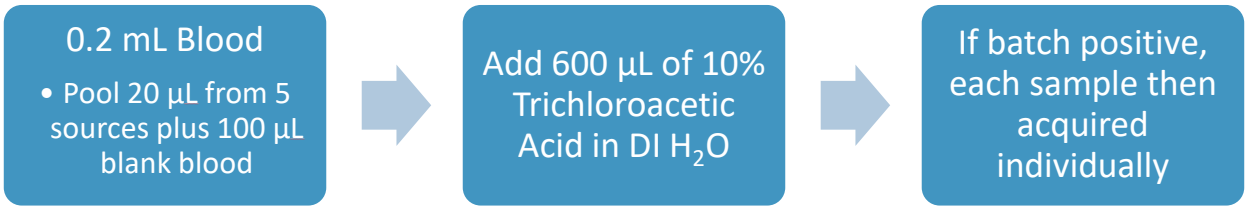

Figure 1. Gabapentin extraction procedure

All samples were received by the original laboratory between January 2020 and December 2021. A total of 2,514 samples were analyzed as part of the project. In total 107 cases (4%) were negative for drugs or alcohol, 1,004 (40%) were positive of alcohol and 1,982 (79%) were positive for drugs. A summary of the distribution of data is provided in Figure 2.

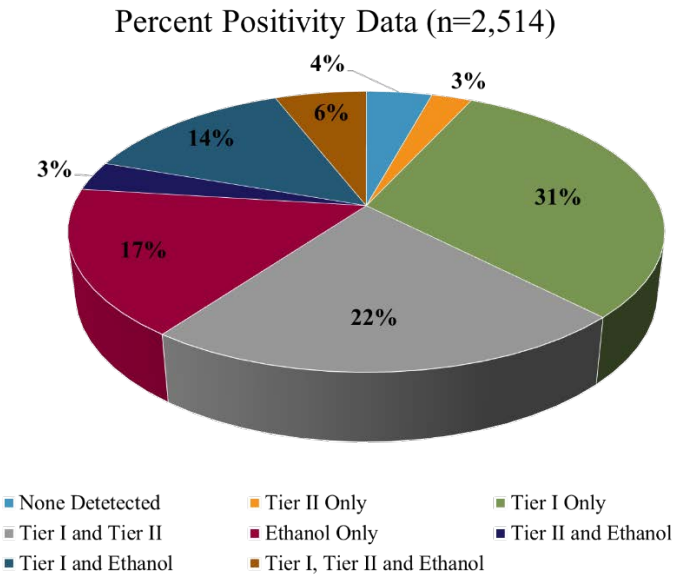

Figure 2. Distribution of Data by Category.

Fifty six percent (56%) of the cases analyzed were positive for a Tier I and/or a Tier II drug only. A summary of the Tier I results is shown below in Table 3. Ethanol was identified in 1,004 cases. The average ethanol concentration was 0.16 g/100 mL (median 0.16 g/100 mL) with a range of 0.01-0.61 g/100mL. THC was identified in 1,227 cases. The average THC concentration was 8.1 (±9.4) ng/mL with a median concentration of 5.2 ng/mL, and a range of 0.5 to 96 ng/mL.

Following THC and ethanol, methamphetamine was the next most frequently detected drug and was identified in 391 cases followed by fentanyl in 348 cases, alprazolam in 87 cases, and cocaine in 86 cases.

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Table 3. Results from Tier I Testing (n=2,514)

Drug	No. of Positive Cases	Positivity (%)
THC	1,227	48.8
Ethanol	1,004	40.0
Methamphetamine	391	15.5
Fentanyl	348	13.8
Amphetamine	347	13.8
Benzoylecgonine	174	6.9
Alprazolam	87	3.5
Cocaine	86	3.4
Methadone	68	2.7
7-Amino Clonazepam	62	2.5
Buprenorphine	52	2.0
Clonazepam	45	1.7
Oxycodone	42	1.6
Tramadol	28	1.1
Morphine	24	0.9
Lorazepam	23	0.9

Provided in Table 4 is the summary for Tier II drugs identified. Of the 2,514 cases analyzed diphenhydramine was the most frequently detected drug (n=187), followed by gabapentin (n=83; analysis was performed on 1,907 cases), hydroxyzine (n=90), 8-aminclonazolam (n=80), fluorofentanyl (n=71) and trazadone (n=69).

Table 4. Results from Tier II Testing (n=2,514 cases).

Drug	No. of Positive Cases	Positivity (%)
Diphenhydramine	187	7.4
Gabapentin*	83	4.3
Hydroxyzine	90	3.5
8-Aminoclonazolam	80	3.1
Fluorofentanyl	71	2.8
Trazodone	69	2.7
Cyclobenzaprine	54	2.1
Doxylamine	53	2.1
Lamotrigine	50	1.9
Etizolam	47	1.8
Eutylone	42	1.6
Mitragynine	34	1.4

*Total number of samples tested for gabapentin is 1,907

In addition to drug positivity, drug combinations were also evaluated. Tier I drugs found in combination with cannabis and ethanol are shown below in Figures 3-4. THC and ethanol were most commonly found with each other (n=359). Outside of ethanol, THC was most identified with CNS stimulants followed by narcotic analgesics (Figure 3). With respect to specific drugs, THC was most commonly found with amphetamine/methamphetamine (n=146) followed by fentanyl (n=118). Ethanol was most commonly identified with CNS Stimulants (n=113), and more specifically with benzoylecgonine in 57 cases followed by amphetamine in 36 cases (Figure 4).

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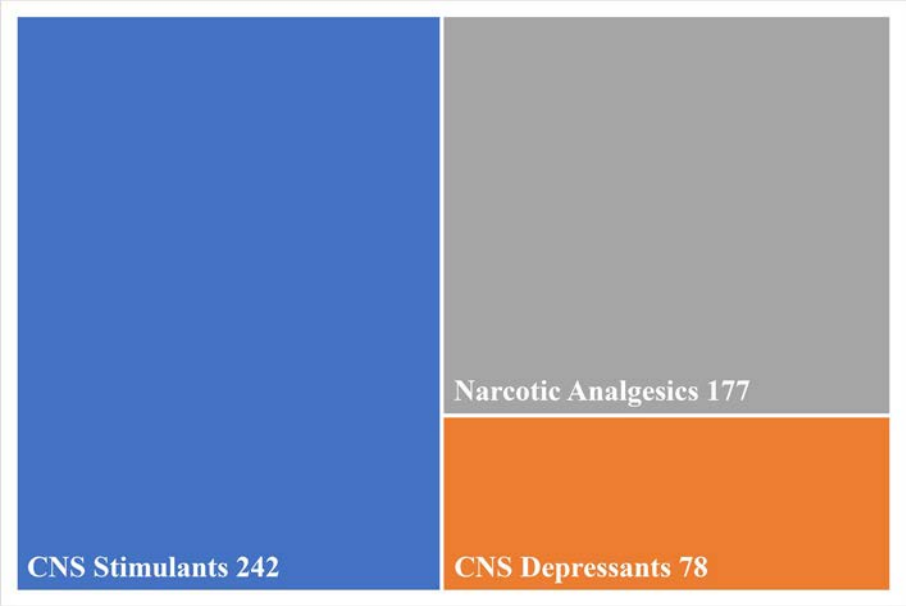

Figure 3. Cannabis found in combination with other Tier I drugs.

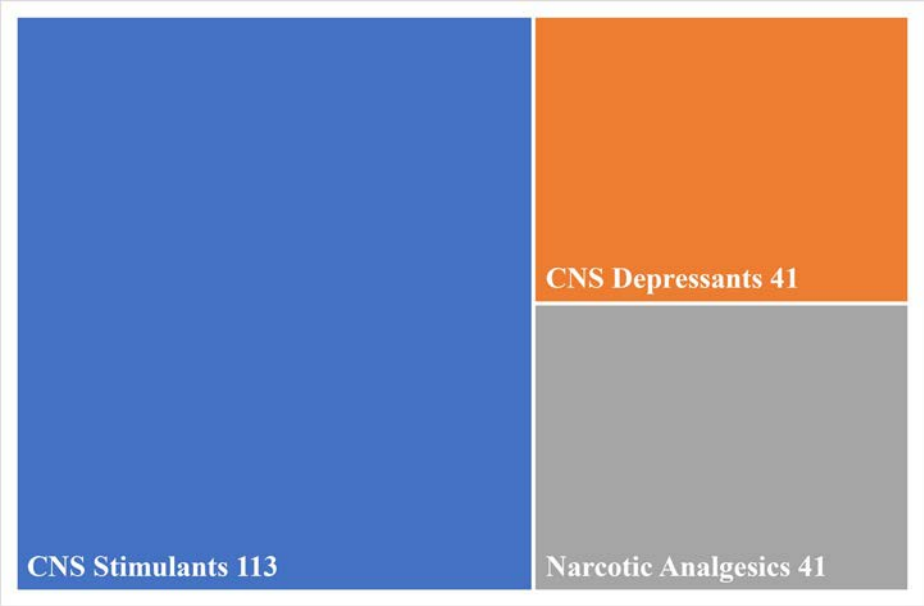

Figure 4. Ethanol found in combination with other Tier I drugs.

Due to the ongoing opioid epidemic centered around fentanyl use and its high rate of positivity (13.8%), drug combinations with fentanyl were evaluated further as shown in Figure 5. Fentanyl was frequently found in combination with CNS stimulants, more specifically methamphetamine (n=131), and THC. With respect to other narcotic analgesics, fentanyl was most frequently identified with methadone (n=48).

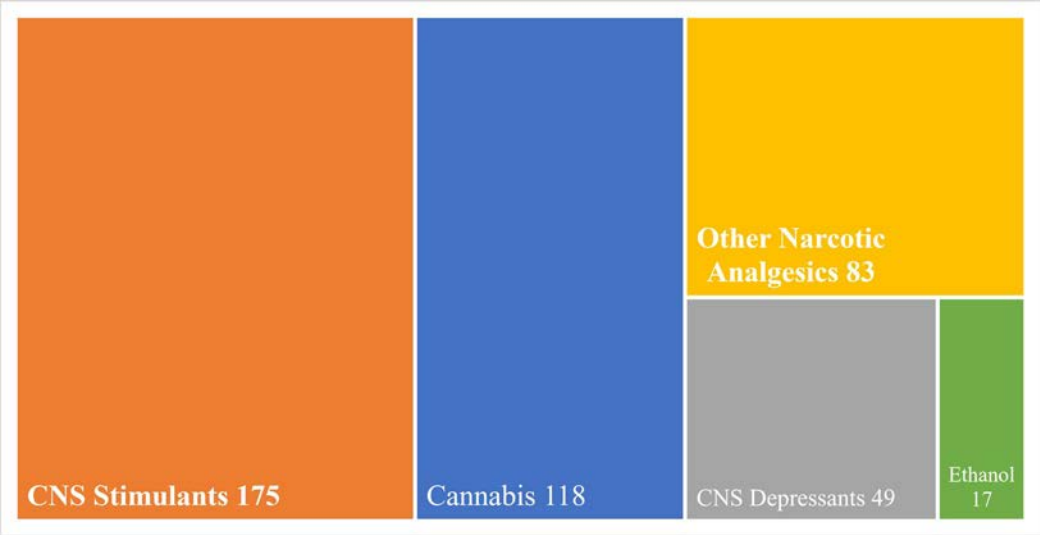

Figure 5. Fentanyl Combinations with other Tier I drugs.

Overall drug positivity for all cases was 79%, nearly double alcohol positivity. When cases that were negative for alcohol and/or drugs are excluded, 24% of all cases analyzed were positive for both drugs and alcohol. The findings of research support the NSC-ADID recommendations for Tier I and Tier II drugs. Drugs identified with the greatest positivity rates are found in Tier I. There were only two Tier I drugs not detected in this data set: alpha-hydroxyalprazolam and oxymorphone. Polydrug use was frequently detected, especially for ethanol and cannabis. Testing for the Tier I recommended scope and ethanol captures 62% of cases with an impairing substance.

One of the challenges associated with getting a comprehensive picture of drug involvement in impaired driving cases are practices which preclude drug testing like stop limit testing. Stop limit testing is the practice of making a determination about whether or not to perform drug testing based on an administratively determined alcohol concentration or the practice of confirming and quantifying only the “most significant” drug identified during screening. The justification for this practice includes the lack of enhanced penalties for combined drug and alcohol use, impairment can be explained by the BAC, limited resources and/or budget, and agency request (14).

With respect to alcohol, 18% of cases were positive for alcohol only and 24% were positive for alcohol and drugs when cases that were none detected are excluded (n=107). Drug positivity was evaluated at various BAC thresholds to assess the impact of drug findings relative to various alcohol concentrations.

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Table 5. Drug Positivity at Various BAC thresholds.

Stop Limit Thresholds				
	<0.08 g/100 mL	**≥0.08 g/100mL**	**≥0.10 g/100 mL**	**≥0.15 g/100 mL**
Tier I Only Positivity	33.0% (n=829)	11.5% (n=288)	10.6% (n=266)	6.4% (n=152)
Tier II Only Positivity	2.9% (n=72)	3.1% (n=79)	2.8% (n=71)	2.1% (n=32)
Tier I and Tier II Positivity	23.9% (n=602)	4.4% (n=111)	3.9% (n=97)	2.7% (n=41)
Positivity for any Tier I, Tier II, or Combo	**60% (n=1,503)**	**19% (n=478)**	**17.3% (n=434)**	**11.1% (n=280)**

Further evaluation was conducted to determine what drugs were identified in these cases. There was a total of 813 cases with the BAC at or greater than 0.10 g/100 mL and 889 cases at BAC at or greater than 0.08 g/100 mL. Seventy-five percent (75%) of labs reported using cutoff thresholds at 0.08 g/100 mL or 0.10 g/100 mL in the 2020 DUID survey (14). Summary data for cases with only a Tier I drug identified at these two thresholds is provided in Table 6.

Table 6. Tier I Drug Findings at Various BAC Thresholds

	≥0.08 g/100 mL		***≥0.10 g/100 mL***	
Drug	**Number of Positive Cases**	**Percent of Cases with Drug**	**Number of Positive Cases**	**Percent of Cases with Drug**
THC	226	25.4%	209	25.7%
BZE	24	2.6%	20	2.4%
Amphetamine	22	2.4%	20	2.4%
Cocaine	15	1.6%	11	1.3%
Methamphetamine	11	1.2%	11	1.3%
Fentanyl	11	1.2%	10	1.2%
Alprazolam	6	0.6%	6	0.7%
7-Aminoclonazepam	4	0.4%	4	0.5%

Stop limit testing is often justified for a number of reasons; however, in the data set analyzed 82% of the cases had drugs identified. At the most commonly used threshold, 0.10 g/100mL, 17.3% of cases are positive for a Tier I and/or Tier II drug. Comparable Tier I positivity rates were observed for the two most commonly used BAC cutoff thresholds. Limiting testing based on alcohol results precludes information of drug involvement in several cases leading to underreporting of drug contributions to impaired driving. Estimates are likely even higher as some samples never even make it to the lab for testing thereby limiting our understanding of the true extent of drug impaired driving.

Objective 2

With respect to NPS, NPS benzodiazepines were seen with the greatest frequency. A method was developed on a Waters Acquity UPLC coupled to Waters Xveo TQ-S Micro triple quadrupole mass spectrometer. Chromatographic separation (Figure 5) was achieved using an Agilent poroshell 3 x 2.7, 100 mm column with 5 mM ammonium formate in deionized water (MPA)

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and 0.1% formic acid in methanol (MPB) with a flow rate of 0.35 mL/minute and a 5 µL injection volume. The column temperature was held at 50 ^{o}C, and the autosampler was held at 15 ^{o}C. The gradient for the method can be found in Table 7.

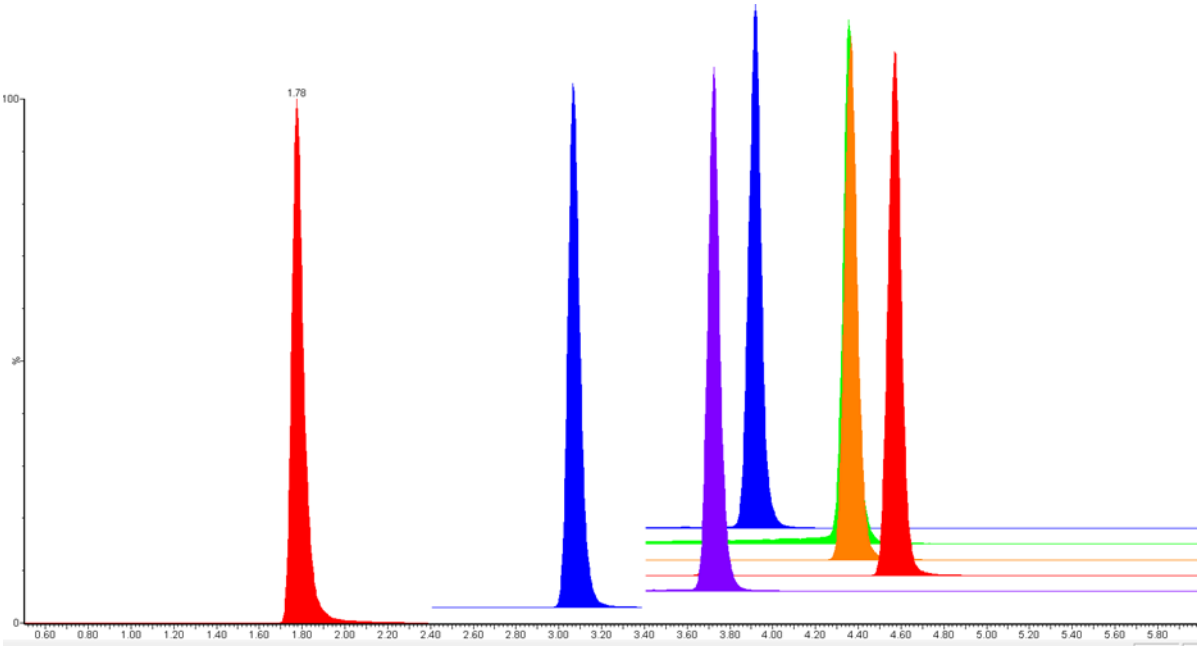

Figure 5. Total ion chromatogram of 50 ng/mL extracted calibrator.

Table 7. UPLC gradient for the analysis of novel benzodiazepines.

Time (min)	% MPB
0	50
5.1	70
5.3	95
5.5	95
5.7	50
6.0	50

The analytes included in this scope included clonazolam, 8-aminoclonazolam, flualprazolam, flubromazolam, bromazolam, etizolam, and flubromazepam.

The mass spectrometer was operated in positive ionization mode for all drugs. MRM transitions, cone voltage, and collision energy for all analytes can be found below in Table 8. Deuterated forms of the targeted drugs were purchased and used as internal standards for all analytes except etizolam. Bromazolam-D5 was used as an internal standard for etizolam.

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Table 8. MS parameters and transitions.

Drug	Precursor ion to quantification ion (m/z) Precursor Ion to qualifier ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
8-aminoclonazolam	324.2 → 296.2 324.2 → 220.1	70 70	26 38
8-minoclonazolam-D4	328.2 → 300.2 328.2 → 224.2	78 78	26 40
Flualprazolam	327.2 → 292.2 327.2 → 223.2	40 40	24 40
Flualprazolam-D4	331.3 → 303.2 331.3 → 227.2	78 78	28 48
Flubromazepam	331.1 → 226.2 331.1 → 104.4	30 30	26 56
Flubromazepam-D4	337.2 → 230.3 337.2 → 105.1	78 78	28 48
Etizolam	343.2 → 314.2 343.2 → 224.1	36 36	24 42
Bromazolam	353.1 → 325.1 353.1 → 205.2	34 34	27 42
Bromazolam-D5	358.2 → 330.2 358.2 → 210.2	66 66	28 42
Flubromazolam	371.1 → 292.2 371.1 → 223.2	54 54	25 42
Flubromazolam-D4	375.1 → 347.2 375.1 → 227.2	54 54	28 42
Clonazolam	354.2 → 308.1 354.2 → 103.1	78 78	27 46
Clonazolam-D4	358.2 → 284.2 358.2 → 103.1	48 48	34 44

A liquid-liquid extraction (LLE) was utilized for analysis. 0.5 mL of blood, 50 µL of internal standard (1 ng/µL) was added along with 0.5 mL of sodium bicarbonate/carbonate (pH 9) and 3 mL MTBE: butyl chloride (60:40). Samples were capped and rotated for ten minutes followed by centrifugation at 3600 rpm for ten minutes. The supernatant was then transferred to a new test tube and dried to completion at 40 ºC for 25-30 minutes. The samples were then reconstituted in 200 µL of 50:50 (MPA:MPB), vortex mixed, and transferred to autosampler vials for analysis.

The limit of detection for all seven drugs was 5 ng/mL. The calibration range for all of the drugs was 5-500 ng/mL. All calibration curves were quadratic using 1/x weighting regression analysis of the peak area of the analyte to the peak area of the internal standard. Validation for this method was carried out over five days and consisted of bias, interference, percent recovery, and limit of quantitation studies. Bias was evaluated on all five days of the study at three different concentration levels, 15, 80, and 400 ng/mL, in triplicate. All three concentrations over the five

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days had bias below 20% for the mean bias as well as for the bias within and between runs. For the interference studies, ten different blood sources were used and showed no interference with the method. For internal standard interference five blood samples with only internal standard and five different blood samples with only the drugs were evaluated. No interferences were found with any of the ten samples. For the commonly encountered analyte interference study, three different samples containing a total of 268 therapeutic and other illicit drugs was used. None of the 268 drugs interfered with the assay. All compounds had percent recovery over 80% with the exception of 8-aminoclonazolam, which had a percent recovery of 35%.

Following the validation, authentic samples that screened positive for a novel benzodiazepine were analyzed on the method. Summary data related to these findings can be found in Table 9. 8-aminoclonazepem was the most frequently identified in the cases and results from the instability of clonazolam. It should be noted that many of these cases were analyzed in excess of six months after submission to the original laboratory, so it is likely that the reported concentrations could be lower than they were at the time of collection.

Table 9. Novel benzodiazepine quantitative summary data.

Drug	Count	Median (ng/mL)	Average (ng/mL)	Std Dev (ng/mL)	Max (ng/mL)	Min (ng/mL)
8-aminoclonazolam	39	11	17	±16	73	5.0
Bromazolam	2	59	59	±75	112	5.5
Clonazolam	2	12	12	±6.6	16	7.3
Etizolam	17	28	44	±32	97	8.4
Flualprazolam	3	6.9	7.4	±1.5	9.2	6.2

Objective 3

Due to the limited of cases where a DRE evaluation was performed that also had a biological sample collected, results for this objective are not included.

Objective 4

The four-year assessment of DUID concentrations included data from 2017 to 2020, and included only Tier I drugs. The results from this review can be seen in Tables 10-14. The total number of cases with quantitative data for 2017 was 15,906 cases, with 13,192 cases in 2018, 17,742 cases in 2019 and 15,250 cases in 2020. There was only data provided for 16,539 of the 2020 cases leaving data from 1,840 (10%) cases out from this assessment. All data from 2020 was calculated using the data that was provided (n=16,539). Ethanol was not tested for in all cases only 9,835 cases in 2017 were screened for ethanol, 10,292 in 2018, 11,292 in 2019, 8,534 in 2020. All data involving ethanol was calculated using the number of cases screened for ethanol and not the total number of cases for that year. All statistical tests, T-test, F-test, and Z-test were evaluated with an alpha level of 0.05. The F-test and T-test were used to determine statistical differences in the average concentration over the four years, and the Z-test was used to determine if there was a statistical difference in the positivity of a drug from year to year. It should be noted that these numbers have not been normalized to account for total case volume.

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With respect to cannabinoids, including delta-9 THC and its metabolites, 11-hydroxy delta-9 THC and delta-9 carboxy THC, there was an increase in positivity over the four years (Table 10). Statistically significant changes in positivity were observed for all three drugs between 2020 compared to 2017. Statistically significant increases in positivity were also note for 11-hydroxy delta-9 THC and delta-9 THC for the years 2018 and 2019 compared to 2017. Though the average concentration for 11-hydroxy delta-9 THC was relatively stable over four year period, there was an increase in average concentration for both delta-9 carboxy THC and delta-9 THC for the years 2019 and 2020, which was determined to be statistically significant relative to concentrations observed in 2017. It should be noted that while statistically significant differences were observed for the average concentrations, the same trend was not observed for median concentrations for any of the three drugs. THC and its metabolites were some of the most commonly encountered drugs over the four-year period being present in over 25% of the DUID cases each year.

Table 10. Cannabinoid percent positivity, count, average concentration, median concentration, min and max concentration by year.

Drug	Year	Positivity (%)	Count (n)	Average (ng/mL)	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)
11-Hydroxy Delta 9 THC	2017	28	5018	3.8	2.8	1.0	53
	2018	30 $^{\alpha}$	5392	3.9	2.9	1.0	49
	2019	32 $^{\alpha, \beta}$	6101	4.0 $^{\alpha}$	2.9	1.0	170
	2020	32$^{\alpha, \beta}$	5444	3.9	2.9	1.0	50
Delta-9 Carboxy THC	2017	45	7951	50	33	5.0	990
	2018	46	8048	51	34	5.0	860
	2019	46	8880	55$^{\alpha, \beta}$	36	5.0	1700
	2020	49 $^{\alpha, \beta}$	8253	57$^{\alpha, \beta}$	39	5.0	800
Delta-9 THC	2017	44	7710	6.4	4	0.5	140
	2018	45 $^{\alpha}$	8023	6.8 $^{\alpha}$	4.4	0.5	100
	2019	47 $^{\alpha, \beta}$	8970	7.1$^{\alpha, \beta}$	4.5	0.5	230
	2020	49$^{\alpha, \beta}$	8110	7.3 $^{\alpha, \beta}$	4.5	0.5	160

α – statistically different than 2017 at alpha level of 0.05.
β – significantly different than 2018 at alpha level of 0.05.

While most CNS stimulants saw some increase in positivity, amphetamine and methamphetamine had the largest increases out of all the stimulants. Amphetamine was found in 11.5% of cases in 2017, which increased to 19.1% in 2020. Methamphetamine was seen in 8.88% of cases in 2017 and 18.1% in 2020. Though out the four years amphetamine stayed the 5th most commonly seen whereas methamphetamine was the 8th in 2017 and the 6th in 2018-2020. MDMA and MDA were never seen at a positivity above 1%. MDMA concentrations have been trending down since 2017 where the median concentration was 326 ng/mL and declined to 150 ng/mL in 2020, although the value difference was determined not to be significant. A similar trend was observed with MDA. Cocaine, benzoylecgonine and cocaethylene positivity was seen at 10% or less across all four years. Average concentrations decreased for most CNS Stimulants, with the exception of methamphetamine which saw an increase in average concentration from 301 ng/mL in 2017 to 381 ng/mL in 2020 (Table 11).

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Table 11. CNS stimulants percent positivity, count, average concentration, median concentration, min and max concentration by year.

Drug	Year	Positivity (%)	Count (n)	Average (ng/mL)	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)
Amphetamine	2017	11	2000	56	36	5.0	1400
	2018	13 α	2351	59	39	5.0	4100
	2019	14 α, β	2754	60 α	39	5.0	5400
	2020	19 α, β	3163	56	37	5.0	2700
Methamphetamine	2017	8.8	1541	301	180	5.0	5500
	2018	11 α	1965	345 α	230	5.4	8800
	2019	12 α, β	2378	376 α, β	240	5.1	8200
	2020	18 α, β	2998	381 α, β	240	5.0	13000
MDMA	2017	0.2	43	326	240	9.4	1300
	2018	0.3	59	209 α	160	6.1	1200
	2019	0.3	67	236	160	5.2	1100
	2020	0.4 α	81	217	150	5.9	1100
MDA	2017	0.2	48	60	25.5	5.2	360
	2018	0.2	49	25 α	14	5.5	230
	2019	0.2	53	22 α	15	6.1	100
	2020	0.4 α, β	69	39	15	5.3	500
Cocaine	2017	4.4	775	108	65	20	7000
	2018	4.5	799	99	66	20	2300
	2019	5.0 α, β	961	109	67	20	7000
	2020	4.1 β	679	90	56	20	1400
Benzoylecgonine	2017	9.7	1686	849	475	50	7800
	2018	10	1791	874	500	50	11000
	2019	9.9	1900	863	510	50	6600
	2020	1	1671	736 α, β	400	50	7200
Cocaethylene	2017	1.3	227	45	37	20	150
	2018	1.1	207	46	36	20	370
	2019	1.3	254	42	36.5	20	130
	2020	0.9 α, β	159	40	36	20	150

α – statistically different than 2017 at alpha level of 0.05.
β – significantly different than 2018 at alpha level of 0.05.

All Tier I drugs within CNS depressants class saw a decrease in positivity over the four-year period (Table 12). This is most notably seen with alprazolam which had a positivity rate of 11.2% in 2017 which decreased to 5.93% in 2020. Along with the decrease in positivity there was also a decrease in average concentration for many of the CNS depressants. As a class overall, CNS depressants had the lowest positivity rate each year which only decreased over the four years.

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Table 12. CNS Depressants percent positivity, count, average concentration, median concentration, min and max concentration by year.

Drug	Year	Positivity (%)	Count (n)	Average (ng/mL)	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)
Alprazolam	2017	11	1951	67	44	5.0	1300
	2018	8.6 [α]	1504	59 [α]	40	5.0	1200
	2019	5.8 [α, β]	1106	49 [α, β]	34	5.0	390
	2020	5.9 [α, β]	981	52 [α, β]	32	5.0	1400
Alpha-Hydroxy Alprazolam	2017	0.8	142	14	7.7	5.0	750
	2018	0.5 [α]	94	8.5	6.8	5.0	36
	2019	0.2 [α, β]	41	24	6.9	5.0	310
	2020	0.2 [α, β]	42	8.3	5.6	5.0	32
Clonazepam	2017	5.6	985	24	16	2.0	350
	2018	4.6 [α]	813	25	18	2.0	270
	2019	3.7 [α, β]	718	23	15	2.0	300
	2020	3.2 [α, β]	538	20 [α, β]	13	2.0	150
7-Amino Clonazepam	2017	5.4	936	35	24	5.0	340
	2018	4.5 [α]	786	34	25	5.0	290
	2019	3.5 [α, β]	668	35	23	5.0	380
	2020	3.1 [α, β]	513	30 [α, β]	21	5.0	180
Lorazepam	2017	2.4	422	48	28	5.0	1000
	2018	1.5 [α]	275	51	31	5.0	380
	2019	1.4 [α]	283	37 [α, β]	22	5.0	350
	2020	1.3 [α]	225	35 [α, β]	21	5.2	480
Diazepam	2017	2.1	372	269	120	20	4200
	2018	1.6 [α]	283	224	100	20	3600
	2019	1.2 [α, β]	239	199 [α]	95	21	1700
	2020	1.0 [α, β]	177	208	88	20	2200
Nordiazepam	2017	2.7	473	237	100	20	2900
	2018	2.1 [α]	377	179 [α]	75	20	4700
	2019	1.6 [α, β]	314	177 [α]	98	20	1800
	2020	1.4 [α, β]	245	201	86	20	2800
Oxazepam	2017	0.5	99	80	37	20	1000
	2018	0.3 [α]	58	54	34	20	440
	2019	0.3 [α]	58	54.4	33	20	440
	2020	0.2 [α, β]	34	84.4	38	20	950
Temazepam	2017	0.7	132	239	67	20	3900
	2018	0.5 [α]	90	154	45	20	1400
	2019	0.3 [α, β]	71	156	56	20	950
	2020	0.2 [α, β]	46	170	65	21	1100
Carisoprodol	2017	0.9	173	4.6	3.7	0.2	18

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	2018	0.5 α	95	4.3	3.0	0.21	18
	2019	0.4 α	86	4.2	3.6	0.21	15
	2020	0.4 α	73	4.2	3.0	0.21	12
Meprobamate	2017	1.1	192	12	11	1.0	48
	2018	0.6 α	114	11	9.4	1.0	42
	2019	0.5 α	96	12	10	1.0	48
	2020	0.5 α	84	10	8.1	1.0	39
Zolpidem	2017	1.2	220	236	155	4.0	1600
	2018	1.0 α	176	298	150	4.1	6500
	2019	0.9 α	181	240	150	4.3	1800
	2020	0.8 α	144	209	135	4.1	1100

α – statistically different than 2017 at alpha level of 0.05.
β – significantly different than 2018 at alpha level of 0.05.

With respect to the class of narcotic analgesics, the number of cases positive for 6-monoacetylmorphine, morphine, and oxycodone steadily declined since 2017 and fentanyl positivity increased from 303 (1.9%) in 2017 to more than 2,100 (13%) in 2020 (Table 13). The differences in positivity between 2017 and 2020 for these five drugs was noted to be statistically significant. Statistically significant increases in positivity were also noted for buprenorphine and its metabolite, norbuprenorphine. With respect to concentrations, there was a downward trend average and median for many drugs in the class, however; average fentanyl concentrations increased from 5.7 ng/mL in 2017 to 9.6 ng/mL in 2020, which was statistically significant. Also of interest the max reported concentration of fentanyl in 2020 was greater than six times higher than what was reported in 2017.

Table 13. Narcotic analgesics percent positivity, count, average concentration, median concentration, min and max concentration by year.

Drug	Year	Positivity (%)	Count (n)	Average (ng/mL)	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)
Codeine	2017	1.3	228	28	8.5	5.0	1200
	2018	0.7 α	135	25	8.0	5.0	620
	2019	0.5 α, β	97	62	8.4	5.0	4300
	2020	0.4 α, β	67	43	10	5.0	470
6-MAM	2017	1.2	223	4.5	2.3	1.0	88
	2018	0.6 α	122	5.7	1.9	1.0	140
	2019	0.5 α	105	5.0	1.6	1.0	270
	2020	0.3 α, β	54	3.1 α	1.9	1.0	15
Buprenorphine	2017	1.1	191	2.1	1.4	0.5	14
	2018	4.4 α	769	1.9	1.3	0.5	24
	2019	3.9 α, β	752	1.9	1.2	0.5	53
	2020	4.2 α	705	1.8 α	1.3	0.5	16
Norbuprenorphine	2017	1.0	183	2.7	1.9	0.5	15
	2018	5.2 α	913	2.0 α	1.3	0.5	44
	2019	4.6 α	879	1.9 α	1.3	0.5	31

	2020	4.9 α	813	1.8 α, β	1.3	0.5	14
Fentanyl	2017	1.9	330	5.7	4.2	0.1	56
	2018	10 α	1776	5.6	3.4	0.1	83
	2019	9.9 α	1886	7.1 α, β	4.6	0.1	140
	2020	12 α, β	2122	9.6 α, β	5.4	0.1	310
Hydrocodone	2017	1.9	330	41	25	5.1	340
	2018	1.2 α	222	32 α	19	5.0	220
	2019	0.9 α, β	183	39 β	27	5.2	180
	2020	1.0 α, β	172	36	25	5.4	330
Hydromorphone	2017	0.5	87	10	2.8	1.0	310
	2018	0.3 α	55	42	2.2	1.0	2100
	2019	0.2 α	52	13	1.8	1.0	400
	2020	0.2 α	35	3.4	1.6	1.0	20
Methadone	2017	2.8	502	247	190	20	1200
	2018	2.5	450	243	215	20	1000
	2019	2.3 α	443	267	230	20	1100
	2020	2.7	448	258	210	20	1200
Morphine	2017	6.7	1179	39	21	5.0	1000
	2018	5.3 α	937	34	67	5.0	1600
	2019	4.6 α, β	881	30 α	15	5.0	1800
	2020	3.5 α, β	581	29 α	15	5.0	870
Oxycodone	2017	4.7	827	65	34	5.0	1200
	2018	3.9 α	689	58	28	5.0	970
	2019	3.0 α, β	578	60	33	5.0	1000
	2020	2.3 α, β	384	56	30	5.0	680
Oxymorphone	2017	1.7	305	5.3	2.1	1.0	130
	2018	1.2 α	216	4.7	2.3	1.0	33
	2019	0.9 α, β	180	3.1 α, β	2.0	1.0	26
	2020	0.5 α, β	99	2.6 α, β	1.7	1.0	25
Tramadol	2017	0.3	63	476	150	26	3400
	2018	1.3 α	233	333	130	21	3000
	2019	0.7 α, β	148	384	190	20	4900
	2020	0.8 α, β	144	258 α	93	20	2300
O-Desmethyltramadol	2017	0.2	41	127	57	21	560
	2018	0.7 α	131	89	61	20	540
	2019	0.5 α, β	100	88	49	20	760
	2020	0.4 α, β	74	79	51	20	530

α – statistically different than 2017 at alpha level of 0.05.
β – significantly different than 2018 at alpha level of 0.05.

Ethanol positivity peaked at 61% in 2019 and declined to 53% in year 2020 (Table 14). Average ethanol concentrations ranged between 155 and 159 mg/dL. Median concentrations ranged

between 154 and 158 mg/dL. The highest average and median concentrations were observed in 2019.

Table 14. Review of Ethanol Concentrations from 2017-2020.

Drug	Year	No. Cases Screened	Positivity (%)	Count (n)	Average (mg/dL)	Median (mg/dL)	Min (mg/dL)	Max (mg/dL)
Ethanol*	2017	9835	59	5812	156	156	10	450
	2018	10127	59	6069	159	158	10	438
	2019	11292	61 α, β	6931	157	155	10	498
	2020	8534	53 α, β	4564	155 β	154	10	457

α – statistically different than 2017 at alpha level of 0.05.
β – significantly different than 2018 at alpha level of 0.05.
* Not all cases were screened for ethanol. Data is based on the cases that were screened for ethanol

Limitations

The population used for the sample analysis (objective 1) and longitudinal concentration assessment (objective 4) were primarily from Pennsylvania and does not give a comprehensive overview of drug population for the entire US population. With respect to drug analysis, there are some Tier II drugs which do not extract well with the procedure used and/or were not within the scope of the method (barbiturates, valproic acid, GHB). Samples were not tested for inhalants. Due to the relatively low frequency with which drugs in Tier II, NPS in particular, were found, additional confirmatory methods were not developed and validated (objective 2).

Another noted limitation of the study was that DRE evaluations were not provided with the samples submitted for analysis; therefore, research into investigating reported signs and symptoms noted by a DRE with toxicologically confirmed drugs was not possible.

Artifacts

Lists of Products

Platform presentation at the 2022 AAFS Annual Meeting entitled “Assessment of the NSC Tier I and Tier II Scope Recommendations in Authentic DUID Cases” by Grace Cieri, February 2022.

Platform presentation at the 2022 IACT Annual Meeting entitled “Drugs and Alcohol: Considerations on Stop Limit Testing in DUI Investigations” by Barry Logan, April 2022.

Platform presentation at the 2022 IDTS Annual Meeting entitled “Assessment of the NSC-ADID Tier I and Tier II Scope Recommendations in Authentic DUID Cases” by Amanda Mohr, August 2022.

Platform presentation at the 2022 SOFT Annual Meeting entitled “Evaluating Drug Positivity for Tier I and Tier II Drugs Relative to BAC Thresholds” by Amanda Mohr, November 2022.

Accepted platform presentation at the 2023 AAFS Annual Meeting entitled “Evaluating Drug Positivity for Tier I and Tier II Drugs Relative to BAC Thresholds” by Grace Cieri, February 2023.

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Accepted platform presentation at the 2023 National Institute of Justice (NIJ) Forensic Science Research and Development (R&D) Symposium entitled "Drug Impaired Driving: A Comprehensive Look at Trends" by Amanda Mohr, February 2023.

Dissemination Activities

Results related to this research have been disseminated at various professional meetings and continuing education courses, which included: 2022 American Academy of Forensic Sciences Annual Meetings, 2022 International Association for Chemical Testing Annual Meeting, 2022 Impaired Driving and Traffic Safety Conference, and 2022 Society of Forensic Toxicologists Annual Meeting. Results from the project were presented during an oral presentation at the Robert F. Borkenstein Conference on "The Effects of Drugs on Human Performance and Behavior," which was held in Philadelphia, PA in both 2021 and 2022. The course is offered to a variety of practitioners including toxicologists and DREs as part of CFSRE's continuing education programs.

Additional dissemination activities are scheduled for 2023 American Academy of Forensic Sciences Annual Meeting and 2023 National Institute of Justice (NIJ) Forensic Science Research and Development (R&D) Symposium. Manuscripts related to the compilation of results of the research, the evaluation of stop limit testing, and evaluation of drug concentration data over four years are being drafted and will be submitted for publication in peer reviewed literature.

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