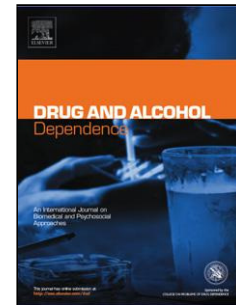


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Fentanyl analog positivity among near-real-time urine drug test results in patients seeking health care

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Highlights

- Fentanyl analogs have emerged as key contributors to drug overdose deaths
- Traditional surveillance methods of drug use are often incomplete and lag in time
- Definitive UDT reveal fentanyl analog positivity in patients seeking health care
- Acetyl fentanyl and 4-ANPP were the most commonly identified fentanyl analogs
- Definitive UDT provide a timely, actionable asset for identifying fentanyl analogs

Abstract: Overdose deaths involving synthetic opioids continue to climb. Fentanyl analogs have been identified as important contributors to these overdoses, but little is known about their prevalence in patients seeking health care. This cross-sectional study of urine drug test (UDT) results from July 15,

2019, through March 12, 2020, included patient specimens analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), submitted by health care professionals as part of routine care to detect fentanyl and fentanyl analogs. A convenience sample approach was used to select patient specimens from diverse health care practices across 50 states, then stratified by fentanyl prescription status. Positivity rates, geographic distribution, and co-occurrence were quantified. The total positivity rate for ten fentanyl analogs was 40.55% in the nonprescribed fentanyl-positive population. The most common fentanyl analogs in this population were 4-ANPP (4-anilino-N-phenethylpiperidine), 30.74%; acetyl fentanyl, 19.40%; and carfentanil, 3.13%. The total positivity rate for four fentanyl analogs was 8.93% in the prescribed fentanyl-positive population, including 4-ANPP, 8.85%; acetyl fentanyl, 0.19%; acryl fentanyl, 0.05%; and 4-FiBF, 0.03%. Counties in Ohio and Kentucky had the highest positivity rates. Acetyl fentanyl and 4-ANPP copositivity occurred in 11.36% of non-prescribed patient specimens. However, acetyl fentanyl and 4-ANPP positivity may not be consistent with fentanyl analog use since both are process impurities, and 4-ANPP is a metabolite of fentanyl. Near real-time, definitive UDT results reveal fentanyl analogs in patients seeking health care, helping clinicians and public health officials better understand their contribution to overdoses and help mitigate the risks they pose.

Abbreviations

UDT urine drug test

LC-MS/MS liquid chromatography–tandem mass spectrometry

IMF illicitly manufactured fentanyl

NPP N-phenethyl-4-piperidone

4-ANPP 4-anilino-N-phenethylpiperidine

DEA Drug Enforcement Administration

STRL Science and Technology Reinvention Laboratory

NFLIS National Forensic Laboratory Information System

4-FiBF 4-fluoroisobutyrylfentanyl

BCI Bureau of Criminal Investigations

Keywords: Carfentanil; 4-ANPP; Drug overdose deaths; Fentanyl; Fentanyl analog; Substance abuse;

Urine drug test

1. Introduction

Between 2017 and 2018, overall drug overdose deaths declined by 4.1% but continue to be the leading cause of injury-related death in the United States (Hedegaard et al., 2020; U.S. Department of Justice Drug Enforcement Administration, 2019). In 2018 alone, 67,367 overdose deaths occurred, largely driven by synthetic opioids other than methadone (Hedegaard et al., 2020). This category primarily includes illicitly manufactured fentanyl (IMF) and its analogs, which together contribute to over 40% of all overdose deaths (Kemp, 2019). Deaths related to fentanyl analogs nearly doubled between July 2016 and June 2017 (O'Donnell et al., 2018). This rapid emergence of fentanyl analogs is especially concerning given the fact that fentanyl-related overdoses already strain the public health system. (O'Donnell et al., 2018).

Fentanyl, a rapid-acting Schedule II synthetic opioid often used in anesthesia and analgesia, is up to 100 times more potent than morphine (AccessScience, 2019; Kemp, 2019). Fentanyl was first synthesized by Paul Janssen in 1959 using benzyl fentanyl and norfentanyl as key precursors in his fentanyl synthesis process (Kemp, 2019; U.S. Department of Justice. Drug Enforcement Administration, 2019). Today, however, the vast majority of legitimate pharmaceutical fentanyl is not synthesized utilizing the Janssen method, but instead uses N-phenethyl-4-piperidone (NPP) and 4-anilino-N-

phenethylpiperidine (4-ANPP or ANPP) as fentanyl precursors (U.S. Department of Justice. Drug Enforcement Administration, 2019). The Janssen method, long considered too sophisticated for chemists working for clandestine laboratories, is instead now the preferred method for producing IMF (U.S. Department of Justice. Drug Enforcement Administration, 2019). According to the Drug Enforcement Administration's (DEA) Science and Technology Reinvention Laboratory (STRL), of the 85 seized fentanyl exhibits evaluated in 2018, 94% were confirmed to be synthesized via the Janssen method (U.S. Department of Justice. Drug Enforcement Administration, 2019). Another popular method of synthesizing IMF is the Siegfried method. The Siegfried method, like legitimate pharmaceutical fentanyl, uses NPP and 4-ANPP as fentanyl precursors, both of which are primarily supplied from Asia (DEA Intelligence Report, 2020). Clandestine labs preferred this method for IMF synthesis until the DEA, in conjunction with the Chinese government, put strict controls on the sale or possession of NPP in 2007 and 4-ANPP in 2010 (U.S. Department of Justice. Drug Enforcement Administration, 2019). In response, makers of IMF who preferred the Siegfried method began using 4-anilinopiperidine as their starting point, as 4-anilinopiperidine can be converted to 4-ANPP through a single chemical step (U.S. Department of Justice. Drug Enforcement Administration, 2019). As a result, the DEA implemented strict control of all benzyl fentanyl (Janssen method) and 4-anilinopiperidine (Siegfried method). Despite these efforts to ban and control certain chemical precursors, IMF and its analogs continue to be a serious threat. In an effort to help law enforcement curb the continued outbreak of fentanyl and its analogs, on February 6, 2020, the U.S. government approved a bill that would continue the temporary scheduling of all fentanyl-related substances for 15 months (GovTrack.us, 2020). Understanding these common precursors, IMF synthesis methods, and metabolism have important clinical implications, as will be described in the discussion.

Since fentanyl is a synthetic compound, the molecule can be modified with relative ease and has led to the creation of fentanyl analogs. A number of these analogs, e.g., carfentanil (Wildnil®), sufentanil

(Sufenta®), and alfentanil (Alfenta®), were developed for use in human and veterinary medicine, while others remain solely contributors to the synthetic opioid crisis (Kemp, 2019). In 2019, fentanyl analogs and other novel synthetic opioids made up nearly 20% of fentanyl, fentanyl-related substances, and other new synthetic opioids seized by law enforcement (Drug Enforcement Administration. Special Testing and Research Laboratory, 2020a). While fentanyl analogs can be created by adapting fentanyl synthesis methods with minor variations, the scope of why and how those techniques are employed by clandestine labs to produce fentanyl analogs remains unclear (Pardo et al., 2019). Some may have been produced to evade or circumvent regulations placed by the DEA and authorities, while others may be synthesized for their high potency. The potency of fentanyl analogs varies widely. For example, the most potent analog, carfentanil, is 10,000 times more potent than morphine, while acetyl fentanyl is 15 times more potent than morphine (Schueler, 2017).

Traditional surveillance of fentanyl and fentanyl analogs largely use post-mortem and drug confiscation data, which can be incomplete and lag in time (harmreduction.org, 2018). These limitations add to the difficulty clinicians and public health officials face when fighting the synthetic opioid crisis, as timely, actionable information is critical when trying to reduce harm and save lives. Using near-real-time urine drug test (UDT) results from July 15, 2019, through March 12, 2020, our analysis provides fentanyl and fentanyl analog positivity rates, geographic distribution, and fentanyl analog co-occurrence in patients seeking health care.

2. Materials and Methods

2.1 Design & Setting

We conducted a cross-sectional study of UDT results from July 15, 2019, through March 12, 2020, of unique patient specimens submitted for testing by health care professionals as part of routine care.

Specimens were collected from health care practices in all 50 states. The analysis used a convenience sample of 300,000 patient specimens with definitive drug testing by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for fentanyl and fentanyl analogs. Each specimen was derived from a unique patient so that no single patient had more than one specimen as part of the analysis. Tests were ordered by a health care professional based on medical necessity. The LC-MS/MS testing method is a laboratory-developed test with performance characteristics determined by Millennium Health, San Diego, California, which is certified by the Clinical Laboratory Improvement Amendments and accredited by the College of American Pathologists for high-complexity testing. This technology is highly sensitive and specific, providing a quantitative identification of parent drugs and their metabolites that is unaffected by other drugs or dietary supplements. LC-MS/MS is the analytical method of choice because of its accuracy and common use in numerous forensic and toxicology laboratories across the nation. For this study, analyte concentrations at or greater than fentanyl, 1 ng/mL; norfentanyl (fentanyl metabolite), 8 ng/mL; 4-ANPP, 2 ng/mL; 4-fluoroisobutyryl fentanyl (4-FiBF), 2 ng/mL; 3-methyl fentanyl, 2 ng/mL; acetyl fentanyl, 2 ng/mL; acetyl norfentanyl (acetyl fentanyl metabolite), 5 ng/mL; carfentanil, 2 ng/mL; butyryl fentanyl, 1 ng/mL; acryl fentanyl, 1 ng/mL; cyclopropyl fentanyl, 1 ng/mL; furanyl fentanyl, 2 ng/mL; methoxyacetyl fentanyl, 2 ng/mL; U-47700, 2 ng/mL; or N-desmethyl U-47700 (U-47700 metabolite), 2 ng/mL were considered positive. Fentanyl, acetyl fentanyl, and U-47700 were considered positive if either the parent analyte or metabolite was positive. All other analogs were considered positive if the parent compound was detected. While U-47700 is not a fentanyl analog, it is a novel synthetic opioid that tends to be grouped with fentanyl analogs based on its similarity in structure and pharmacologic effects (Armenian et al., 2017). Fentanyl analog tests were only performed for the fentanyl and/or norfentanyl-positive population. This testing strategy is based on extensive analytical and clinical validation performed at Millennium Health, where it was determined that fentanyl analogs are found in fewer than 0.11% of samples negative for fentanyl. The fentanyl analogs selected for

evaluation include those most commonly identified in the U.S. at the time of testing validation (U.S. Department of Justice. Drug Enforcement Administration, 2018). The study protocol was approved by the Aspire Independent Review Board and includes a waiver of consent for the use of de-identified data. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2 Statistical Analysis

Fentanyl positivity rates were calculated for the entire sample population. Fentanyl analog positivity rates were calculated for the fentanyl-positive population. Positivity rates were further stratified for both the fentanyl prescribed and non-prescribed populations. Order forms completed by the health care professionals included an option to report the patient's prescribed fentanyl. Clopper-Pearson 95% binomial CIs were calculated for all positivity rates. In addition to individual analog rates, positivity rates for an "Analog Subset" were calculated based on detection of any analog, except 4-ANPP, acetyl fentanyl, and carfentanil. The Analog Subset was designed to measure the contribution of less common fentanyl analogs. We also calculated the "Total Analog Set" positivity, which evaluates all fentanyl analogs. All rates and confidence intervals are expressed as a percentage.

Fentanyl, 4-ANPP, acetyl fentanyl, carfentanil, and the "Analog Subset" positivity rates were also analyzed based on the geographic location of the patient's home address zip code and alignment to U.S. counties and states. For the county-level analyses, fentanyl and fentanyl analog positivity is based on the non-prescribed fentanyl-positive population only.

A 1-sided Fisher's exact test was used to assess whether analog pairs were co-occurring more frequently than expected based on the underlying positivity rates of the individual analogs. This co-positivity analysis was performed on the non-prescribed fentanyl positive population only.

R statistical software version 3.5.0 (R Project for Statistical Computing) was used for data analysis.

Statistical significance was set at P less than .05.

3. Results

3.1 Demographics and Samples

In a sample of 300,000 unique UDT patient specimens received between July 15, 2019, and March 12, 2020, a total of 295,647 specimens from individual patients not prescribed fentanyl were analyzed. An additional 4,353 specimens from patients prescribed fentanyl were analyzed for the same time period (Table 1). The median age (interquartile range) of the fentanyl non-prescribed population was 49 (23-75) years, and 54.02% were women. The median (interquartile range) age of the fentanyl-prescribed population was 61 (45-77) years, and 64.28% were women. These differences likely reflect the use of fentanyl for analgesia in the population prescribed fentanyl, as does the fact that 92.06% of patient specimens in the fentanyl-prescribed population came from pain management or primary care practices compared to 54.10% coming from these practice specialties in the group not prescribed fentanyl (Table 1). Other factors potentially influencing demographic differences include that women tend to be prescribed opioids at a higher rate than men, and older adults are more likely to experience chronic pain, which also increases the likelihood of being prescribed an opioid (Campbell et al., 2010). The fentanyl positivity rate for the population not prescribed fentanyl was 4.00% (95% CI, 3.93-4.07) and 85.96% (95% CI, 84.90-86.98) for the population prescribed fentanyl.

3.2 Fentanyl and fentanyl analog positivity rates

The total positivity rate for ten fentanyl analogs was 40.55% in the non-prescribed fentanyl-positive population (Table 1, "Total Analog Set"). The positivity rates for the five most common fentanyl analogs

in this population were 4-ANPP, 30.74% (95% CI, 29.91%-31.58%); acetyl fentanyl, 19.40% (95% CI, 18.69-20.13); carfentanil, 3.13% (95% CI, 2.83-3.46); acryl fentanyl, 0.59% (95% CI, 0.46-0.75); and butyryl fentanyl, 0.27% (95% CI, 0.19-0.38) (Table 1). The total positivity rate for the four most common fentanyl analogs was 8.93% (95% CI, 8.03-9.88) in the prescribed fentanyl-positive population. The positivity rates for these analogs were 4-ANPP, 8.85% (95% CI, 7.95-9.80); acetyl fentanyl, 0.19% (95% CI, 0.08-0.39); acryl fentanyl, 0.05% (95% CI, 0.01-0.19); and 4-FiBF, 0.03% (95% CI, 0.0-0.15) (Table 1). The Analog Subset (all analogs except 4-ANPP, acetyl fentanyl, and carfentanil) positivity rate in the non-prescribed fentanyl-positive population was 1.24% (95% CI, 1.05-1.46) and 0.08% (95% CI, 0.02-0.23) for the prescribed fentanyl-positive population (Table 1).

3.3 Non-prescribed fentanyl and fentanyl analog positivity by U.S. county

For the non-prescribed fentanyl-positive population, fentanyl and fentanyl analog-positive specimens were widely distributed throughout the U.S., but the distribution varied for each analog (Figures 1A-1E). There were 181 counties across 30 states that had at least 10 fentanyl positive samples. Acetyl fentanyl and 4-ANPP shared a similar geographic distribution as fentanyl (Figures 1B and 1C). Acetyl fentanyl was detected in 154 counties across 25 states, and 4-ANPP was detected in 175 counties across 29 states. The counties with the highest positivity rates for acetyl fentanyl and 4-ANPP were located in Ohio and Kentucky, with a few exceptions. In these counties, 4-ANPP positivity ranged from 48.42% (95% CI, 38.04-58.90) in Boone, KY to 76.19% (95% CI, 52.83-91.78) in Miami, OH (Table 2). Acetyl fentanyl positivity rates ranging from 38.65% (95% CI, 31.98-45.65) in Montgomery, OH to 61.90% (95% CI, 38.44-81.89) in Estill, KY (Table 2). Carfentanil positivity is much more geographically limited, being detected in 37 counties from five states (Figure 1D). Notably, 19 of the top 20 counties for carfentanil positivity were found in Ohio, with the top 10 counties all located within northeastern Ohio (Table 2). Carfentanil positivity ranged from 5.56% (95% CI, 0.14-27.29) in Pasco, FL to 50.00% (95% CI, 28.22-71.78) in Portage, OH (Table 2). The Analog Subset group had a more diverse geographic distribution than

carfentanil, but the overall total positivity rates were lower (Figure 1E). The Analog Subset group was detected in 56 counties across 16 states.

3.4 Fentanyl analog co-occurrence

For the non-prescribed fentanyl-positive population, the number of fentanyl analogs found in combination with fentanyl ranged from zero to four. For 59.45% of this population, no fentanyl analogs were identified (Table 3). For the remaining 40.55% of non-prescribed fentanyl-positive patients, one to four fentanyl analogs co-occurred with fentanyl. In 12.78% of patients, more than one fentanyl analog was present, and every fentanyl analog co-occurred with another fentanyl analog in at least one of these patients. The combination of 4-ANPP and acetyl fentanyl was the only combination present in more than 10% of patients. In fact, no other combination of fentanyl analogs was present in more than 1% of patients (Table 3). Acetyl fentanyl and 4-ANPP co-occurred in 11.36% of patients, a number that significantly exceeded expectation (Fisher's test, $p < .001$). Pairwise combinations involving 4-ANPP or acetyl fentanyl were also found to be significantly enriched for carfentanil, acryl fentanyl, butyryl fentanyl, 4-FiBF and cyclopropyl fentanyl. Other unique pairwise combinations significantly enriched included acryl fentanyl with butyryl fentanyl, 4-FiBF with methoxyacetyl fentanyl, and cyclopropyl fentanyl with methoxyacetyl fentanyl (Table 3).

4. Discussion

This study reveals fentanyl analog positivity for 10 fentanyl analogs in a fentanyl-prescribed and non-prescribed population. Acetyl fentanyl and 4-ANPP were identified more often than all other fentanyl analogs combined for both populations. This finding is not necessarily surprising, given that 4-ANPP is the only analog tested that is a minor metabolite, via amide hydrolysis, of pharmaceutical-grade fentanyl, IMF, and several fentanyl analogs, such as acetyl fentanyl, methoxyacetyl fentanyl, butyryl

fentanyl, 4-FiBF, and acryl fentanyl (Wilde et al., 2019). Four-ANPP is also a process impurity in the synthesis of both pharmaceutical-grade and illicit fentanyl (Schueler, 2017). As discussed in the introduction, the Siegfried method of fentanyl synthesis utilizes 4-ANPP as the preferred fentanyl precursor, though the U.S. government has taken action to limit its availability (U.S. Department of Justice. Drug Enforcement Administration, 2019). Despite this action, 4-ANPP is consistently one of the top fentanyl analogs detected in IMF-related drug confiscations (Drug Enforcement Administration. Special Testing and Research Laboratory, 2020a). Alternatively, 4-anilinopiperidine may be used to synthesize 4-ANPP and ultimately IMF (U.S. Department of Justice. Drug Enforcement Administration, 2019). The involvement of 4-ANPP in the synthesis of IMF and legitimate pharmaceutical fentanyl, as well as its role as a minor metabolite of fentanyl and several tested fentanyl analogs, likely explain the majority of 4-ANPP's 30.74% positivity rate in the non-prescribed fentanyl-positive population and the 8.85% positivity rate in the fentanyl-prescribed population. Acetyl fentanyl's positivity in the fentanyl-prescribed and non-prescribed populations is likely because it, too, is a known process impurity in the manufacture of pharmaceutical-grade fentanyl and IMF and like 4-ANPP, is also commonly found in IMF-related drug confiscations (Drug Enforcement Administration. Special Testing and Research Laboratory, 2020a; Schueler, 2017; World Health Organization, 2015). Our data demonstrates that acetyl fentanyl and 4-ANPP share a similar geographic distribution as fentanyl (Figures 1B and 1C), and the combination of 4-ANPP and acetyl fentanyl was the only fentanyl analog combination present in more than 10% of patients. This evidence points to the presence of acetyl fentanyl and 4-ANPP primarily as process impurities, but with fentanyl and several fentanyl analogs metabolism to 4-ANPP also playing a role. Lastly, there is a remote possibility that acetyl fentanyl and 4-ANPP may be added directly to IMF, though both analogs are much less potent than fentanyl (Schueler, 2017; Wilde et al., 2019), and we are unaware of any evidence that support this possibility.

Appreciating that 4-ANPP and/or acetyl fentanyl positivity may not be consistent with illicit fentanyl use is especially relevant for the clinician prescribing fentanyl. Clinicians should refrain from immediately accusing a patient of using IMF when definitive UDT results identify 4-ANPP and/or acetyl fentanyl in a patient prescribed fentanyl, since both analogs are potential process impurities of pharmaceutical-grade fentanyl, and 4-ANPP is a minor metabolite of fentanyl. A more likely interpretation of a positive 4-ANPP would be consistent with prescribed fentanyl use and not use of IMF. However, due to the low prevalence of acetyl fentanyl (0.19%) in the prescribed fentanyl population, caution should be observed when interpreting this as consistent with prescribed fentanyl. This is not the case, however, with other fentanyl analogs identified in this study. Based on our current understanding of the literature, the positive findings for acryl fentanyl and 4-FiBF, in the fentanyl-prescribed population, likely represents the use of IMF or use of a counterfeit medication sold as a prescribed opioid, for example, but actually containing acryl fentanyl or 4-FiBF as part of its contents (Centers for Disease Control, 2018).

These possibilities also explain the fentanyl analogs identified in the non-prescribed fentanyl population. In this population, carfentanil had the highest positivity rate at 3.13%. This is especially troubling given carfentanil's potency, which is 10,000 times more potent than morphine and greatly increases the risk of drug overdose (O'Donnell et al., 2017). Geographically, carfentanil did not exhibit a wide distribution throughout the U.S. like acetyl fentanyl and 4-ANPP, but instead, its presence was limited almost exclusively to Ohio, with the highest rates in northeast Ohio. These carfentanil findings are consistent with the Ohio Attorney General's Bureau of Criminal Investigations (BCI) data, which analyzes approximately 40% of confiscated drugs in Ohio. In the first half of 2019, BCI identified six counties in northeast Ohio that contributed to 62% of carfentanil found in Ohio (harmreduction.org, 2019). Those counties were Summit, Ashtabula, Portage, Trumbull, Medina, and Mahoning, all of which ranked in the

top 20 for carfentanil positivity in this study (Table 2). This is an important finding and points to the role near-real time, definitive UDT can play in drug surveillance.

The “Analog Subset” positivity rates were low compared to 4-ANPP, acetyl fentanyl, and carfentanil; however, their importance should not be minimized. From July 2016 through December 2018, acryl fentanyl and cyclopropyl fentanyl were both implicated in over 700 deaths, while other “Analog Subset” fentanyl analogs, like butyryl fentanyl, have been identified as important contributors to drug overdose deaths (O’Donnell et al., 2020).

Finding co-occurring fentanyl analogs in this population of patients seeking health care is important. The CDC found that between July and December of 2016, nearly half of fentanyl analog-related deaths involved two or more fentanyl analogs or fentanyl, or both (O’Donnell et al., 2017). This study identifies fentanyl analog combinations in 12.78% of patients, and one or more fentanyl analogs with fentanyl in over 40% of patients. These numbers speak to the risk of fentanyl analog-involved overdose that exists in these patients and offer an important piece of information clinicians can use to steer conversations with their patients that are positive for these analogs. Acetyl fentanyl and 4-ANPP found together or with other fentanyl analogs were the most commonly identified combinations (Table 3). While the presence of acetyl fentanyl and 4-ANPP may be consistent with IMF use, other combinations involving analogs, like acryl fentanyl and butyryl fentanyl, were more common than anticipated. Beyond improving our awareness of dangerous drug combinations, knowledge of these combinations may yield important clues as to dealer and user preferences, IMF synthesis variability, illicit drug supply, and perhaps drug supply routes and warrant further investigation.

4.1 Limitations and considerations

Several limitations of the current study should be considered. Our analysis relies on accurate and complete reports of prescribed medications by ordering clinicians. It is possible that the fentanyl non-prescribed population contains individuals expected to be positive for prescribed fentanyl in cases with missing report information. This could inflate the fentanyl positivity rate in the non-prescribed population, although the impact on the current study is expected to be minimal. For most fentanyl analogs, positivity was based on detection of the parent molecule because they are highly abundant in urine and considered the most suitable target for testing (Wang and Bernert, 2006; Watanabe et al., 2017). While the major route of metabolism for fentanyl and fentanyl analogs is via oxidative *N*-dealkylation to form each analog's unique "nor" metabolite, the only metabolites included for testing were norfentanyl (fentanyl metabolite), acetyl norfentanyl (acetyl fentanyl metabolite), and *N*-desmethyl U-47700 (U-47700 metabolite) because these metabolites are also highly abundant in urine. It should be noted that 4-ANPP is considered a minor metabolite, via amide hydrolysis biotransformation, of fentanyl and several fentanyl analogs, which may influence its positivity (Wilde et al., 2019). We also recognize that while our results were compiled from samples collected across all 50 states in multiple healthcare settings, we did not attempt to normalize the distribution according to geographic region. This may have influenced the geographic presentation of results and it may not reflect the entire population using IMF and fentanyl analogs. It is possible that we do not have the power to detect fentanyl and fentanyl analogs in certain geographical locations. The current investigation also requires that samples be positive for fentanyl and/or norfentanyl in order for UDT testing to be performed for fentanyl analogs. It is possible that we have underestimated fentanyl analog detection within the sample population, despite our internal validation and an additional similar study showing a low percentage of fentanyl analog positivity without fentanyl and/or norfentanyl being present (Goggin et al., 2018). In aligning patient home zip code to U.S. counties, we recognize that this may not perfectly reflect the location of fentanyl use or UDT specimen collection. While information

regarding the window of detection for fentanyl analogs is limited, intravenous formulations of fentanyl and norfentanyl has been established to be detected in urine for up to 48 hours and up to 96 hours, respectively (Baselt, 2011). Our dataset is also limited to data collected from July 15, 2019, to March 12, 2020, and therefore, the scope of fentanyl analog discovery represents a defined 8-month period. Furanyl fentanyl was not detected in this study, despite reports that it was one of the more commonly found fentanyl analogs in drug seizures prior to 2019. However, seizure data collected during this study period show furanyl fentanyl is no longer among the top analogs seized (Drug Enforcement Administration. Special Testing and Research Laboratory, 2020b, 2020c). Additionally, this study did not include testing for benzyl fentanyl, which is a known precursor to the creation of fentanyl via the Janssen synthesis method, because it is not commonly identified by law enforcement (U.S. Department of Justice Drug Enforcement Administration, 2019). Fentanyl analog manufacture, distribution, and patterns of use are quickly evolving. The results discussed in the current study may not reflect drug use trends prior to the study period.

5. Conclusions

This data is the first that formally investigates fentanyl analog positivity in UDT in a population of patients seeking health care. Our data is comprised of patient samples sent to us by clinicians utilizing definitive UDT as part of routine patient care. The significance of this for clinicians is that there is an opportunity to intervene, to explain the risks of overdose with fentanyl that may be combined with one or more fentanyl analogs, and to understand which substances are present with the hopes of initiating harm reduction measures. Additionally, clinicians should recognize the presence of acetyl fentanyl and/or 4-ANPP is not necessarily indicative of fentanyl analog use since these are both process impurities, and 4-ANPP is a metabolite of fentanyl. However, this data suggest that clinicians may have

fentanyl analog users within their practice, and knowledge of these fentanyl analogs will help providers make more informed care decisions.

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Conflict of Interest Disclosures:

Drs. Stanton, LaRue, Bundy, Dawson, Huskey and Mr. Whitley are employees of Millennium Health, LLC, San Diego, California.

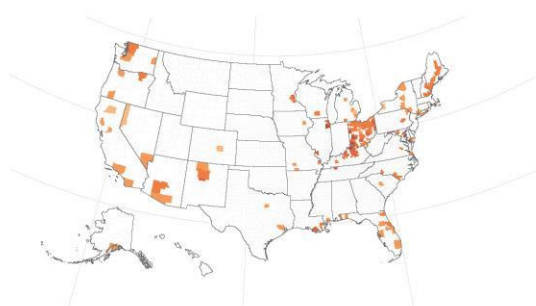
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A. Fentanyl



B. 4-ANPP



C. Acetyl Fentanyl



D. Carfentanil



E. Analog Subset

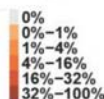


Figure 1. Non-Prescribed Fentanyl and Fentanyl Analog Positivity Rates by U.S. County. Fentanyl, 4-ANPP, acetyl fentanyl, carfentanil and the Analog Subset positivity rates are shown for U.S. counties. The analog subset measures positivity for any of the tested fentanyl analogs with the exception of 4-ANPP, acetyl fentanyl and carfentanil. Only counties with at least 10 fentanyl positive patient specimens are displayed. Fentanyl positivity was calculated for the entire non-prescribed population. Fentanyl analog positivity was only calculated for the fentanyl positive population due to the reflex testing method used in the current study.

Table 1. Characteristics of Urine Specimens Tested for Fentanyl and Fentanyl Analogs Between July 15, 2019 and March 12, 2020

| Characteristics | Fentanyl Non- Prescribed Population | Fentanyl Prescribed Population |
|---|--|---|
| unique requisitions (% of total patient specimens, n=300,000) | 295,647 (98.55%) | 4,353 (1.45%) |
| female requisitions (%) | 159,701 (54.02%) | 2,798 (64.28%) |
| median age [IQR] | 49 [23-75] | 61 [45-77] |
| <i>Specialty of the referring health care practice</i> | | |
| Addiction Medicine (%) | 19,416 (6.57%) | 7 (0.16%) |
| Behavioral Health (%) | 40,565 (13.72%) | 59 (1.36%) |
| Multispecialty and Other (%) | 24,229 (8.20%) | 250 (5.74%) |
| OBGYN (%) | 2,661 (0.90%) | 0 (0.00%) |
| Pain Management (%) | 100,035 (33.84%) | 3,224 (74.06%) |
| Primary Care Physician (%) | 59,553 (20.14%) | 786 (18.06%) |
| Treatment Center (%) | 49,188 (16.64%) | 27 (0.62%) |
| <i>Fentanyl Positivity Rates [95% CI] in the Total Sample Population *</i> | | |

| | | |
|--|---------------------|---------------------|
| Fentanyl | 4.00 [3.93-4.07] | 85.96 [84.90-86.98] |
| <i>Fentanyl Analog Positivity Rates [95% CI] in the Fentanyl Positive Population **</i> | | |
| 4-ANPP | 30.74 [29.91-31.58] | 8.85 [7.95-9.80] |
| Acetyl Fentanyl | 19.40 [18.69-20.13] | 0.19 [0.08-0.39] |
| Carfentanil | 3.13 [2.83-3.46] | n.d. |
| Acryl Fentanyl | 0.59 [0.46-0.75] | 0.05 [0.01-0.19] |
| Butyryl Fentanyl | 0.27 [0.19-0.38] | n.d. |
| 4-FiBF | 0.20 [0.13-0.30] | 0.03 [0.00-0.15] |
| Cyclopropyl Fentanyl | 0.08 [0.04-0.16] | n.d. |
| Methoxyacetyl Fentanyl | 0.08 [0.04-0.16] | n.d. |
| U-47700 | 0.05 [0.02-0.11] | n.d. |
| 3-Methyl Fentanyl | 0.01 [0.00-0.05] | n.d. |
| Furanyl Fentanyl | n.d. | n.d. |
| Total Analog Set *** | 40.55 [39.66-41.44] | 8.93 [8.03-9.88] |

| | | |
|----------------------|------------------|------------------|
| Analog Subset. ***** | 1.24 [1.05-1.46] | 0.08 [0.02-0.23] |
|----------------------|------------------|------------------|

Abbreviations: not detected in the study population (n.d.)

Notes:

All positivity rates and 95% CI values are expressed as percentages.

* Fentanyl positivity rates (%) are based on the prescribed or non-prescribed total sample populations.

** Fentanyl analog positivity rates(%) are based on the prescribed or non-prescribed fentanyl positive populations.

*** The 'Total Analog Set' positivity rates are based on detection of any fentanyl analog tested for in the current study.

**** The 'Analog Subset' positivity rates are based on detection of any fentanyl analog tested for in the current study with the exception of 4-ANPP, acetyl fentanyl and carfentanil.

Table 2. Top 20 US Counties Ranked by Analog Positivity Rate in the Non-Prescribed Fentanyl Positive Population

| R a n k | 4-ANPP | | Acetyl Fentanyl | | Carfentanil | | Analog Subset * | |
|------------------|------------------------|----------------------------|------------------------------|----------------------------|----------------------|----------------------------|-----------------------|---------------------------|
| 1 | Miami , OH | 76.19 [52.83- 91.78] | Estill, KY | 61.90 [38.44- 81.89] | Porta ge, OH | 50.00 [28.22- 71.78] | Penobs cot, ME | 11.36 [3.79- 24.56] |
| 2 | Clark, OH | 69.23 [38.57- 90.91] | Baltim ore City, MD | 55.56 [30.76- 78.47] | Sum mit, OH | 46.24 [38.91- 53.68] | Muhle nberg, KY | 10.00 [0.25- 44.50] |
| 3 | Bourb on, KY | 63.64 [30.79- 89.07] | Mason , KY | 50.00 [21.09- 78.91] | Ashla nd, OH | 31.58 [12.58- 56.55] | Owen, KY | 9.52 [1.17- 30.38] |
| 4 | Montg omery , OH | 63.29 [56.32- 69.86] | Ottaw a, OH | 50.00 [29.93- 70.07] | Wayn e, OH | 25.00 [13.64- 39.60] | Broom e, NY | 9.09 [0.23- 41.28] |
| 5 | Hamilt on, OH | 60.42 [55.33- 65.34] | Woodf ord, KY | 50.00 [21.09- 78.91] | Stark, OH | 23.89 [18.49- 30.00] | Green e, OH | 8.33 [1.03- 27.00] |
| 6 | Brown , OH | 58.82 [32.92- 81.56] | Owen, KY | 47.62 [25.71- 70.22] | Ashta bula, OH | 23.61 [14.40- 35.09] | Mason , KY | 8.33 [0.21- 38.48] |
| 7 | Sando val, NM | 58.82 [32.92- 81.56] | Muski ngum, OH | 47.52 [39.05- 56.09] | Cuya hoga, OH | 20.13 [14.11- 27.34] | Somer set, MD | 8.33 [0.21- 38.48] |

| | | | | | | | | |
|----|------------------------------|----------------------------|---------------------|----------------------------|------------------------|---------------------------|-------------------------------|--------------------------|
| 8 | Green e, OH | 58.33 [36.64- 77.89] | Clark, OH | 46.15 [19.22- 74.87] | Tusca rawa s, OH | 18.18 [5.19- 40.28] | Palm Beach, FL | 7.14 [0.18- 33.87] |
| 9 | Woodf ord, KY | 58.33 [27.67- 84.83] | Miami , OH | 42.86 [21.82- 65.98] | Medi na, OH | 16.67 [6.37- 32.81] | Portsm outh City, VA | 7.14 [0.18- 33.87] |
| 10 | Baltim ore City, MD | 55.56 [30.76- 78.47] | Will, IL | 42.86 [17.66- 71.14] | Lorai n, OH | 15.79 [9.12- 24.70] | Hillsbo rough, FL | 6.45 [0.79- 21.42] |
| 11 | Jeffers on, OH | 55.17 [35.69- 73.55] | Carroll , KY | 42.42 [25.48- 60.78] | Craw ford, OH | 15.38 [1.92- 45.45] | Frankli n, KY | 5.88 [0.72- 19.68] |
| 12 | Saint Clair, IL | 52.94 [27.81- 77.02] | Grant, KY | 42.11 [26.31- 59.18] | Lake, OH | 14.71 [4.95- 31.06] | Lake, OH | 5.88 [0.72- 19.68] |
| 13 | Bullitt, KY | 52.38 [29.78- 74.29] | Hamilt on, OH | 41.67 [36.69- 46.78] | Huro n, OH | 14.08 [6.97- 24.38] | Baltim ore City, MD | 5.56 [0.14- 27.29] |
| 14 | Ross, OH | 50.94 [42.91- 58.95] | Brown , OH | 41.18 [18.44- 67.08] | Trum bull, OH | 13.95 [8.48- 21.15] | Medin a, OH | 5.56 [0.68- 18.66] |
| 15 | Camp bell, KY | 50.00 [42.38- 57.62] | Boone , KY | 41.05 [31.06- 51.62] | Guer nsey, OH | 13.04 [2.78- 33.59] | Pasco, FL | 5.56 [0.14- 27.29] |
| 16 | Grant, KY | 50.00 [33.38- 66.62] | Harris on, KY | 40.00 [16.34- 67.71] | Richl and, OH | 12.59 [7.51- 19.39] | Terreb onne, LA | 5.56 [0.14- 27.29] |

| | | | | | | | | |
|----|------------|------------------------|----------------|------------------------|--------------|-----------------------|----------------|----------------------|
| 17 | Mason, KY | 50.00 [21.09-78.91] | Campbell, KY | 39.20 [31.95-46.83] | Mahoning, OH | 10.71 [6.12-17.06] | Gallatin, KY | 5.26 [0.13-26.03] |
| 18 | Will, IL | 50.00 [23.04-76.96] | Clermont, OH | 38.97 [30.73-47.70] | Erie, OH | 9.23 [3.46-19.02] | Nelson, KY | 5.00 [0.13-24.87] |
| 19 | Kenton, KY | 49.05 [42.86-55.26] | Wood, OH | 38.89 [27.62-51.11] | Defiance, OH | 5.88 [0.15-28.69] | Schuylkill, PA | 4.76 [0.12-23.82] |
| 20 | Boone, KY | 48.42 [38.04-58.90] | Montgomery, OH | 38.65 [31.98-45.65] | Pasco, FL | 5.56 [0.14-27.29] | Cook, IL | 4.55 [0.95-12.71] |

Notes:

Only counties with at least 10 fentanyl positive patient specimens (i.e. at least 10 fentanyl analog tests) were analyzed.

For each Fentanyl analog evaluated the 20 counties with the highest positivity are displayed.

* The 'Analog Subset' positivity rates are based on detection of any fentanyl analog tested for in the current study with the exception of 4-ANPP, acetyl fentanyl and carfentanil.

Table 3. Pairwise Co-Detection of Fentanyl Analogs in the Non-Prescribed Fentanyl Positive Population

| | 4-ANPP | Acetyl fentanyl | Carfentanil | Acryl fentanyl | Butyryl fentanyl | 4-FiBF | Cyclopropyl fentanyl | Methoxyacetyl fentanyl | U-47700 | 3-Methyl fentanyl |
|------------------------|--------------|-----------------|-------------|----------------|------------------|--------|----------------------|------------------------|---------|-------------------|
| 4-ANPP | 3,632 | | | | | | | | | |
| Acetyl fentanyl | 1,342** * | 2,292 | | | | | | | | |
| Carfentanil | 136*** | 92** | 370 | | | | | | | |
| Acryl fentanyl | 66*** | 56*** | 0 | 70 | | | | | | |
| Butyryl fentanyl | 31*** | 21*** | 3 | 4*** | 32 | | | | | |
| 4-FiBF | 17*** | 9* | 2 | 0 | 0 | 24 | | | | |
| Cyclopropyl fentanyl | 7* | 7*** | 0 | 0 | 0 | 0 | 10 | | | |
| Methoxyacetyl fentanyl | 5 | 2 | 1 | 0 | 0 | 1* | 1** | 10 | | |
| U-47700 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | |
| 3-Methyl fentanyl | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

Notes:

- A total of 11,814 fentanyl analog tests were performed (i.e. fentanyl positive samples). 7,024 (59.45%) were negative for all fentanyl analogs tested.
- The number of times two different fentanyl analogs were detected together is displayed. Comparisons against oneself (the diagonal) reflect the total number of times a given analog was detected.
- A 1-sided Fisher's Exact Test was performed for all pairwise comparisons. In this context, the Fisher's test was used to determine if the two analytes were detected together more often than expected based on the individual drug positivity rates.
- Fisher's Exact Test P values: * p<0.05, **p<0.01, ***p<0.001