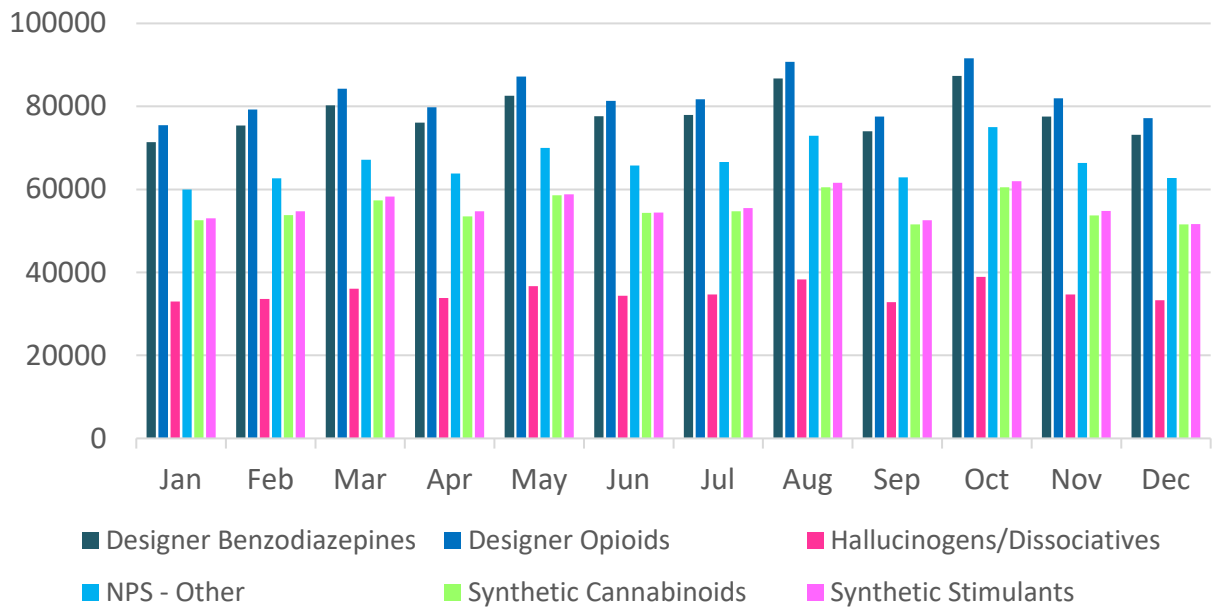
Clinical Update:

NOVEL PSYCHOACTIVE SUBSTANCES 2024: A YEAR IN REVIEW

New or Novel Psychoactive Substances (NPS) are a diverse group of synthetic substances created to mimic the effects of prescription or illicit drugs that are often used non-medically.[1] The term NPS doesn't necessarily mean that the substance is novel, only that it is newly observed in the illicit drug supply. There are various classes of NPS including designer opioids, designer benzodiazepines, synthetic cannabinoids, synthetic stimulants, hallucinogens/dissociatives, and others. NPS may change frequently as legislation to control specific chemical structures or classes of NPS is introduced. Once an NPS has been deemed a controlled substance, often new or modified, non-regulated NPS appear. This remains a challenge for regulatory and enforcement agencies, monitoring institutions, clinical and toxicology laboratories, as well as healthcare providers. It is important to point out that some substances that we refer to as NPS may be approved for use in other countries or approved for veterinary use. The focus of this clinical update is to evaluate changes observed in the prevalence of NPS detected at Aegis in 2024. There were two updates to Aegis NPS testing in 2024, the first in April and the second in December.

Aegis offers NPS testing by class in both urine and oral fluid. Test offerings include designer opioids, designer benzodiazepines, synthetic cannabinoids, synthetic stimulants, hallucinogens/dissociatives, and NPS – Other. **Figure 1** shows 2024 NPS order rates by class and by month.

Figure 1. 2024 NPS order rates by class



The designer opioids class was the most frequently ordered NPS class followed closely by designer benzodiazepines (~95% of designer opioids). The NPS-Other class was the next most frequently ordered class at ~80% of designer opioids. Order rates for synthetic cannabinoids and synthetic stimulants were very similar (~67 and 68% respectively) and the hallucinogens/dissociatives class was the least frequently ordered class (~42% of designer opioids). When evaluating NPS data it is important to consider that order rates may impact NPS detection and observed prevalence both within a class and between classes. For example, in August of 2024 there were

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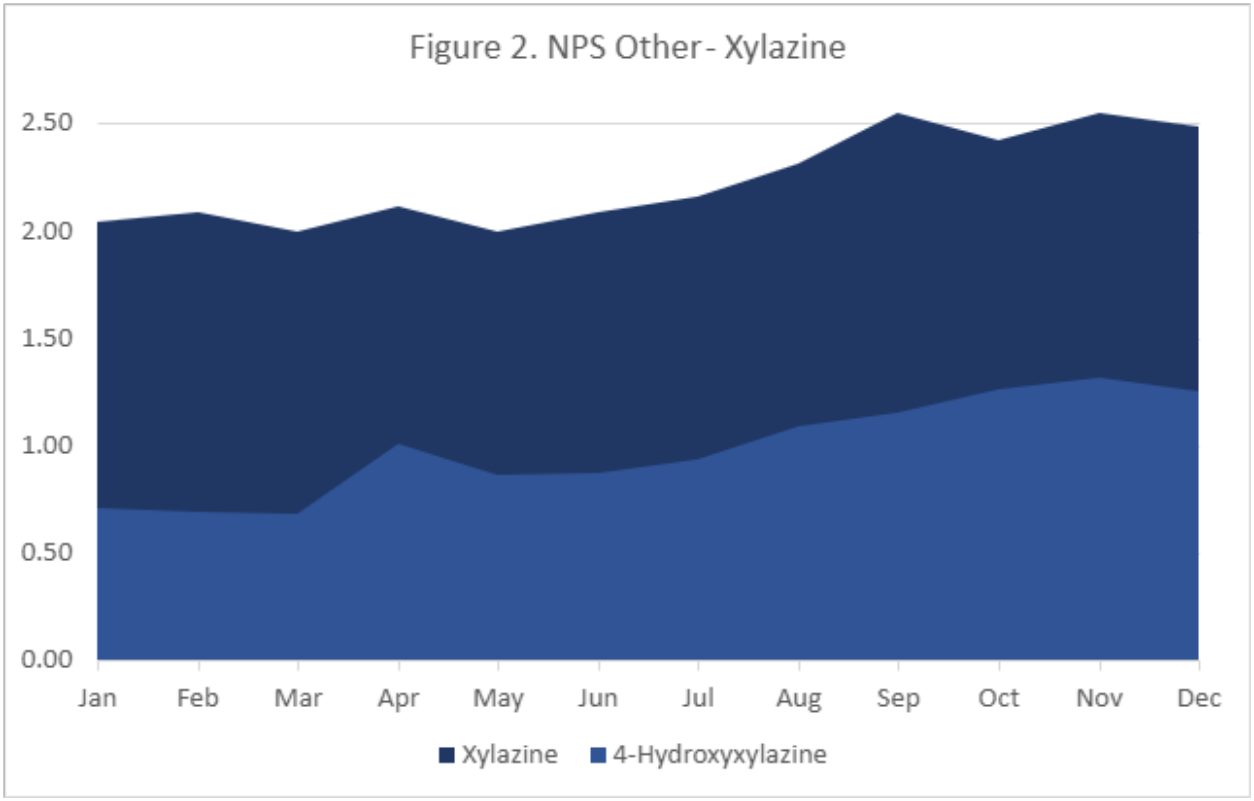
approximately 15,000 more tests for designer opioids than in January. Additionally, the hallucinogens/dissociatives class had the lowest positivity rates, but also had the lowest order rates. In order to lessen the impact of differences in order rates, data presented in this clinical update reflects percent positivity (i.e. # of positives/# of class orders by month*100).

NPS-OTHER

The NPS-Other category includes substances that do not easily fit within a designated NPS classification. This group currently consists of xylazine, medetomidine, phenibut, tianeptine (also known as gas station heroin), as well as BTMPS (also known as bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate or Tinuvin® 770) and phenylbutazone which were added to Aegis NPS testing in December. Some of these compounds are not specifically NPS but rather are veterinary medications that are emerging adulterants in the illicit drug supply. Xylazine is an alpha-2 adrenergic receptor agonist approved for veterinary use as a sedative with analgesic and muscle relaxant properties. It is not approved for use in humans and may cause unique withdrawal episodes and severe necrotic skin ulcers. In November of 2022, the U.S. Food and Drug Administration (FDA) released an alert regarding risks to patients exposed to xylazine in the illicit drug supply.[2] The prevalence of xylazine and its metabolite in 2024 is shown in **Figure 2**.



Arguably the most interesting story of NPS at Aegis in 2023 was xylazine as its detection continued to increase from 2022 and it became the most prevalent NPS detected at Aegis in 2023 irrespective of NPS class. Xylazine continued to increase throughout 2024 and although the order rates for the NPS - Other class are less than some other NPS classes, xylazine comfortably remains the most predominant NPS detected at Aegis irrespective of NPS classification.

Prevalence of additional compounds in the NPS-Other category in 2024 are shown in **Figure 3**. Like xylazine, medetomidine is an alpha-2 adrenergic receptor agonist and has been detected as an adulterant in the illicit drug supply.[3] Unlike xylazine, medetomidine has been approved for medical use by the FDA and is available in both

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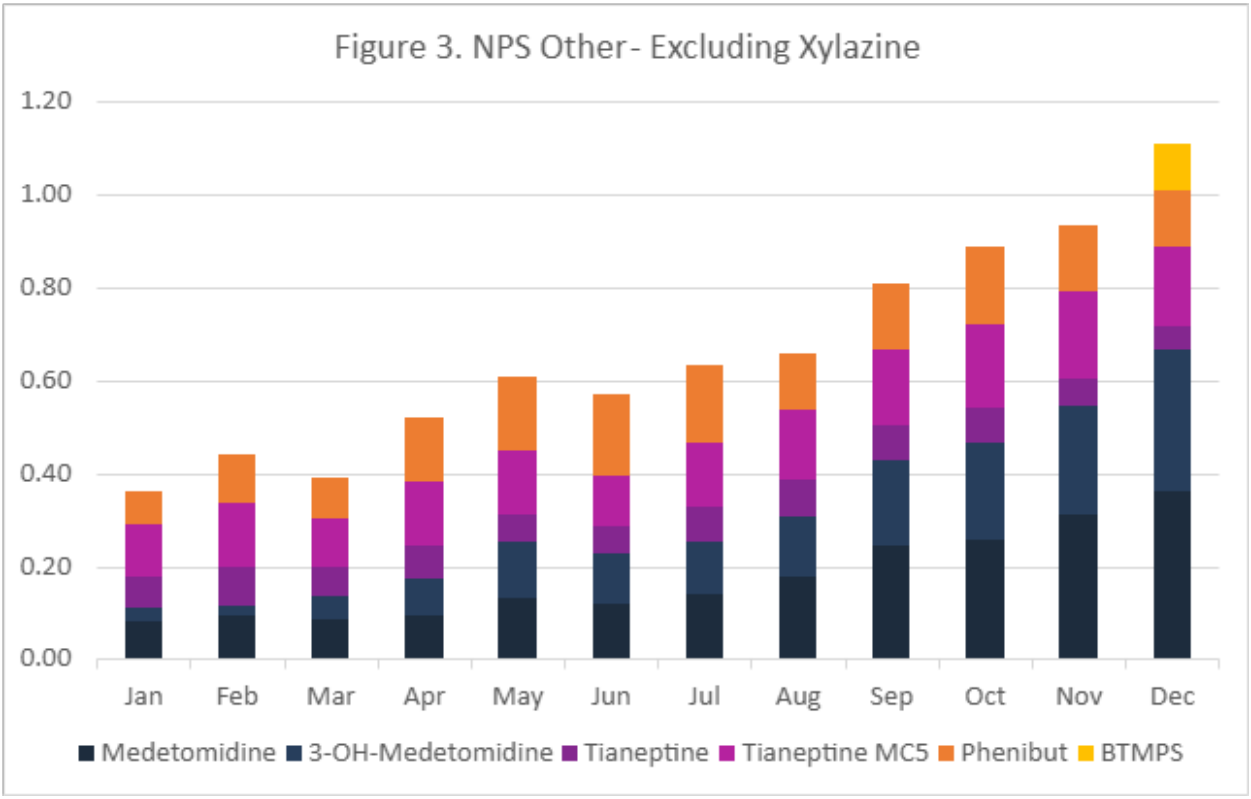
veterinary and human pharmaceutical formulations. Medetomidine and its metabolite 3-hydroxy medetomidine were added to NPS testing in September of 2023. In December of 2023, the Center for Forensic Science Research and Education (CFSRE) issued a Toxic Adulterant Alert for medetomidine.[4] By May of 2024, CFSRE released a public alert indicating that medetomidine was rapidly proliferating across the United States.[5] Throughout 2024, positivity of medetomidine and its metabolite in Aegis testing increased by 350% and 933% respectively. 97% of samples positive for medetomidine and/or its metabolite were also positive for xylazine.



BTMPS is the latest emerging adulterant in the illicit drug supply. It is an industrial chemical that has been used in the manufacture of plastics and is a potent Ca2+ channel blocker.[6] BTMPS rapidly appeared in the illicit drug supply in August of 2024 and has already been identified as a public health concern.[7,8] BTMPS was added to Aegis NPS-Other testing in December of 2024, and its detection was nearly that of phenibut. Phenibut is a synthetic form of gamma aminobutyric acid (GABA) that acts similarly to benzodiazepines. Its positivity increased by 142% from January to a mid-year maximum but then decreased somewhat to end the year with an overall 71% increase in positivity compared to January. Tianeptine is structurally classified as an atypical tricyclic antidepressant but at high doses it has mu opioid receptor activity. Thus, misuse can result in opioid-like highs, and users can develop tolerance and dependence. The FDA reported tianeptine products have been linked to serious adverse events including overdose and death.[9] In Aegis testing, tianeptine metabolite MC5 positivity increased by 33% from the first half of 2024 compared to the second half.

DESIGNER OPIOIDS

Designer opioids include various subclasses of compounds such as fentanyl analogs or “fentalogs,” along with “nitazene analogs,” and others. The top designer opioids detected in 2024 are shown in **Figure 4**. The most prevalent designer opioid, fluorofentanyl, is often detected with despropionyl fluorofentanyl which may be either a metabolite of fluorofentanyl or a process impurity. Fluoro norfentanyl, a metabolite of fluorofentanyl, was added to Aegis testing in April. Fluorofentanyl has three positional isomers (meta-, ortho- and para-) that are not distinguished in Aegis’ designer opioids test. The para-fluorofentanyl isomer has been associated with overdose

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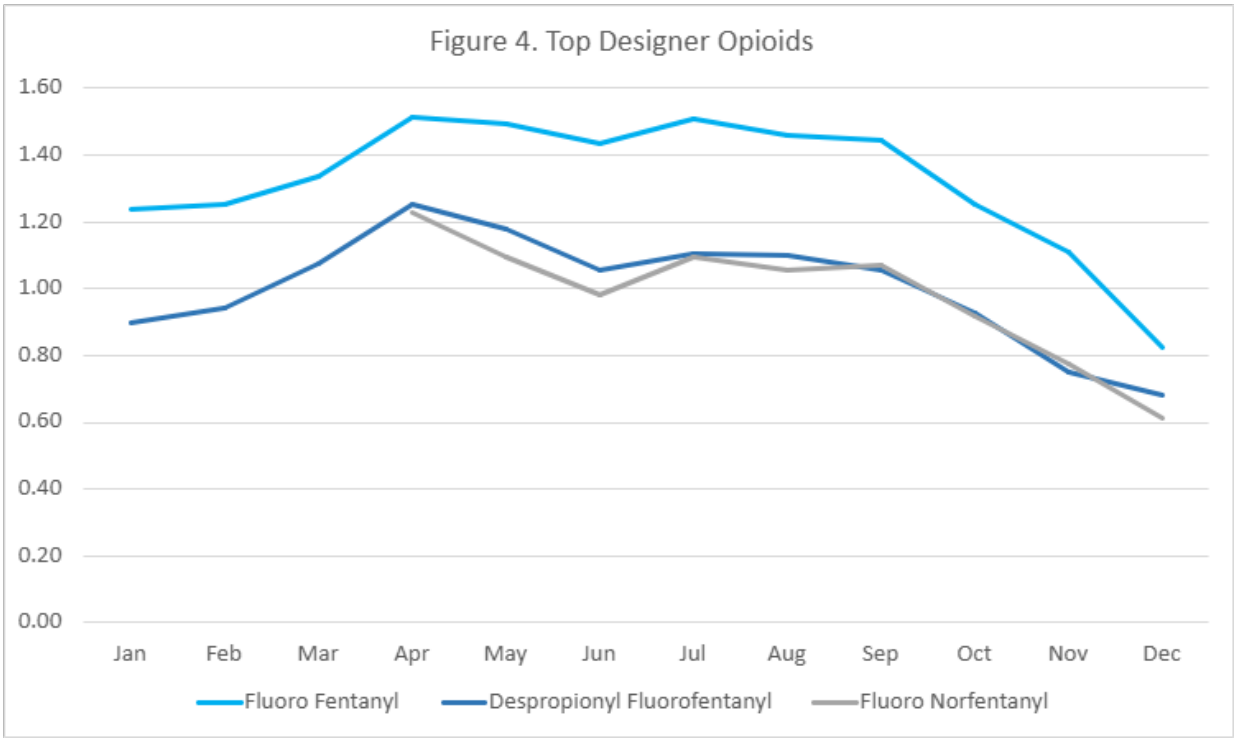
deaths.[10,11] For multiple years fluorofentanyl was the most frequently detected NPS at Aegis among all NPS classes until April of 2023 when xylazine overtook it as the most prevalent NPS detected at Aegis irrespective of NPS class. Fluorofentanyl positivity in 2024 increased by nearly 22% in Q1 and remained fairly steady until Q4 when its positivity decreased by 43%. Despite this decrease in positivity, fluorofentanyl was the second most prevalent NPS detected at Aegis in 2024, irrespective of NPS class. Despropionyl fluorofentanyl detection followed that of fluorofentanyl but averaged approximately 75% of its detection level and fluoro norfentanyl positivity was very similar to that of despropionyl fluorofentanyl.



In 2019, there were class-wide bans on fentanyl-related substances in both the United States and China that impacted the availability of this type of designer opioid.[12] In 2022, a rise in detection of "nitazene" compounds ocurred, with 4-hydroxy nitazene becoming the next most prevalent designer opioid marker detected at Aegis, followed closely by metonitazene. This trend continued in 2023 but changed slightly in 2024. Positivity of nitazene designer opioids in 2024 is shown in **Figure 5**. N-desethyl metonitazene, a metabolite of metonitazene, was added to Aegis testing in April. The positivity of this metabolite ranged from 134 to 238% of metonitazene positivity, and despite not being included in testing for the entire year, its annual positivity was slightly greater than that of 4-hydroxy nitazene. Thus, N-desethyl metonitazene was the next most prevalent designer opioid after fluorofentanyl and its related compounds, followed closely by 4-hydroxy nitazene, and then by metonitazene. 4-hydroxy nitazene has been identified as a universal metabolite of nitazene analogs containing a 5-nitro group, N,N-diethylamine and an associated phenyl ether.[13] This includes isotonitazene, metonitazene, etonitazene, protonitazene and butonitazene. In Aegis testing, 4-hydroxy nitazene has predominantly been detected alone or in combination with metonitazene but it has also been detected with other nitazene analogs.

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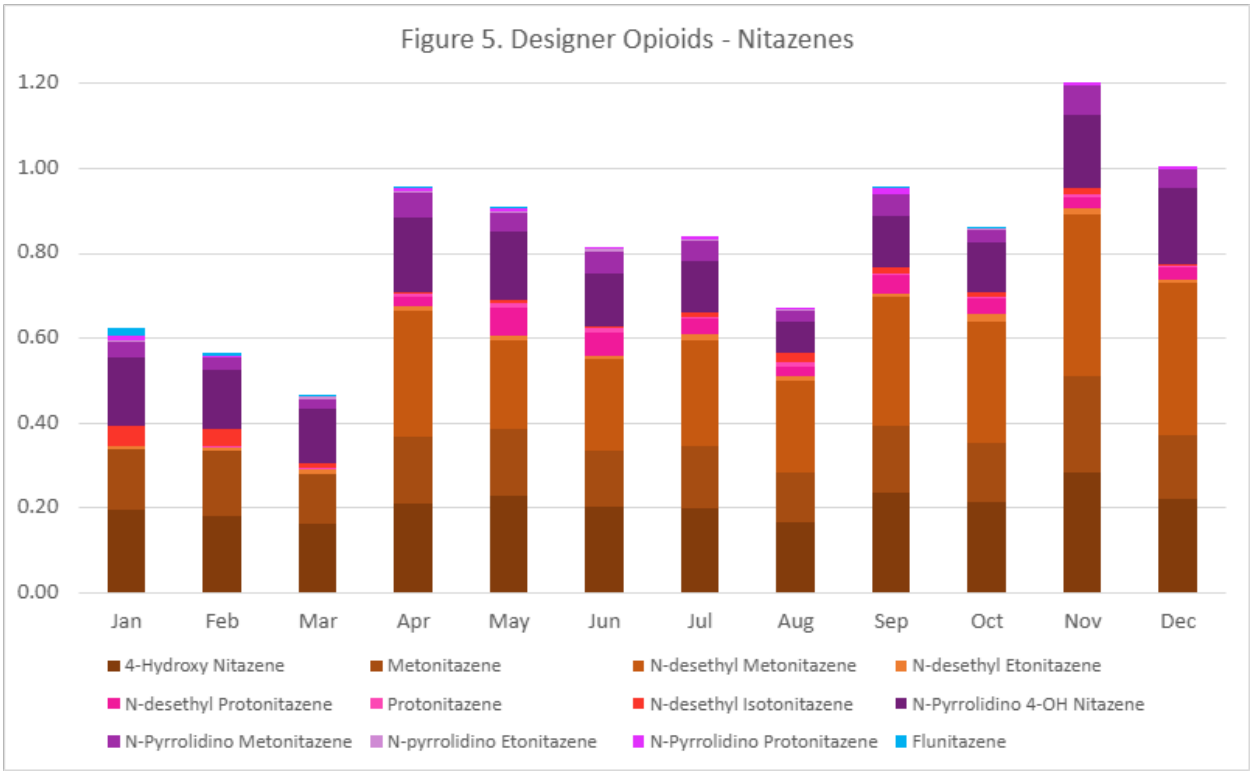

The next most prevalent designer opioid in 2024 was N-Pyrrolidino 4-OH Nitazene, which was added to Aegis testing in September of 2023. Its positivity fluctuated throughout the year, decreasing from April to August but then increasing for the remainder of the year. It is likely that as 4-hydroxy nitazene is a universal metabolite for nitazene compounds with certain structural characteristics that N-Pyrrolidino 4-OH Nitazene will be a universal metabolite for the N-Pyrrolidino form of those same nitazenes including metonitazene, etonitazene, and protonitazene. Of the parent N-Pyrrolidino compounds, N-Pyrrolidino 4-OH Nitazene was most frequently detected with N-pyrrolidino metonitazene but was also detected in combination with N-pyrrolidino metonitazene and N-pyrrolidino protonitazene as well as with N-pyrrolidino etonitazene and N-pyrrolidino protonitazene alone.

The positivity of other fentanyl related designer opioids is shown in **Figure 6**. Of these, Valeryl Fentanyl carboxy metabolite was the most prevalent and had an annual positivity slightly less than N-Pyrrolidino 4-OH Nitazene. Its positivity fluctuated throughout the year but was at its highest in Q4 with December positivity increasing 175% relative to January positivity. The next most prevalent designer opioid was norcarfentanil, which is a shared metabolite of carfentanil and remifentanil. Detection of norcarfentanil was one of the more interesting results in designer opioid detection in 2023 as its detection was minimal throughout the year but increased significantly in Q4 of 2023. In 2024, detection was highest through April but then decreased sharply and fluctuated at lower levels for the remainder of the year. Acryl Fentanyl and Benzyl Fentanyl were the next most prevalent designer opioids in 2024. Both had their lowest positivity in Q1 but increased to peak in June then decreased and held similar positivity through the remainder of the year. Despropionyl Bromofentanyl was opposite in that its detection which was highest in Q1 significantly decreased and had minimal detection in the second half of 2024. Brorphine was mainly detected in the first four months of the year.

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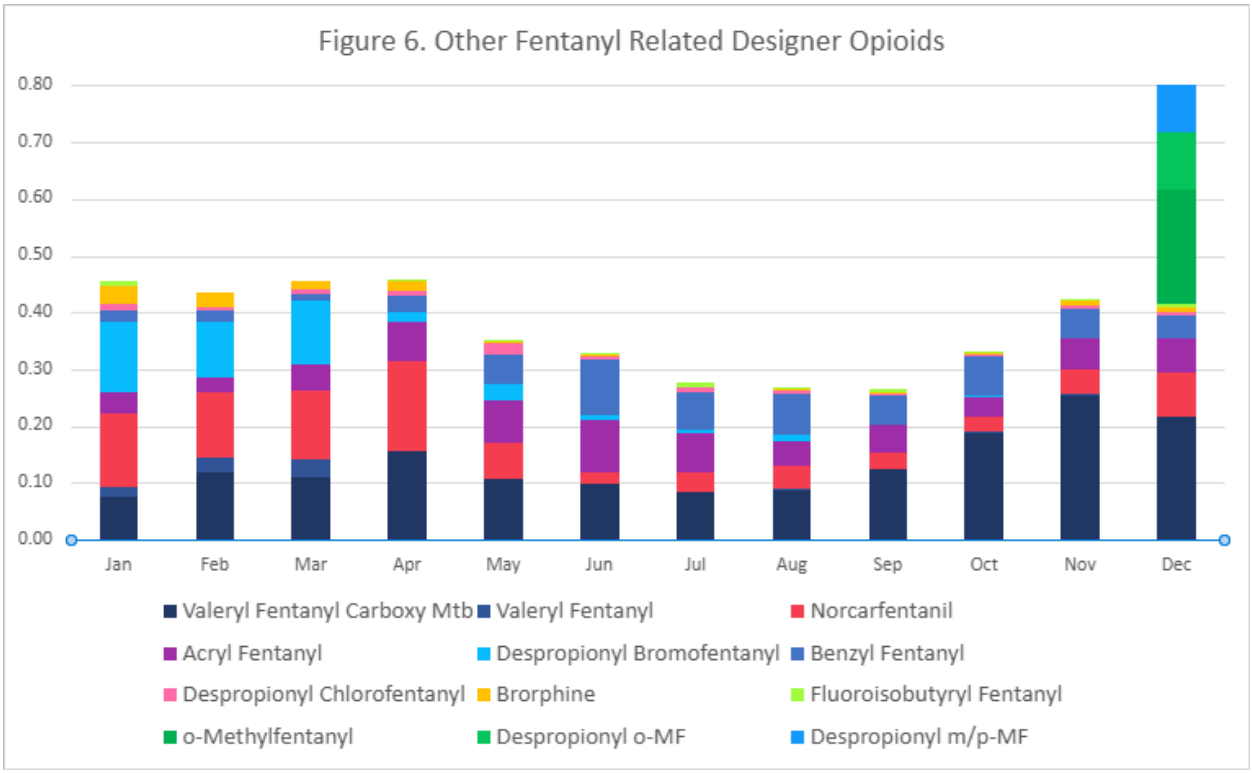

Methylfentanyl, which has 3 positional isomers (ortho-, meta- and para-) was newly added to Aegis' designer opioid testing in December of 2024. In our testing, the ortho- isomer is distinguished from the meta- and para-isomers, which are not distinguished from one another. In December, CFSRE released a public alert noting that ortho-methylfentanyl, the newest synthetic opioid identified in fatal drug overdoses, is proliferating across North America.[14] Considering only December designer opioid positivity, ortho-methylfentanyl positivity was approximately 90% that of valeryl fentanyl carboxy metabolite and 4-hydroxy nitazene positivity which were the same. Designer opioids with less than 20 detections in the year were not included in the graphs.

DESIGNER BENZODIAZEPINES

The positivity of the top designer benzodiazepines detected in 2024 is shown in **Figure 7**. In December of 2022, the DEA temporarily placed clonazolam, etizolam, flualprazolam, flubromazolam and diclazepam in Schedule I of the Controlled Substances Act (CSA) to limit access to these substances.[15] However, bromazolam remained unscheduled. As a result, in 2023, bromazolam became the most prevalent designer benzodiazepine detected, surpassing clonazolam which is detected mainly via its metabolite 8-aminoclonazolam. In 2024, bromazolam detection showed a slight decreasing trend with bromazolam and metabolite positivity decreasing by almost 37 and 19% respectively from January to December, and although bromazolam remained the most prevalent designer benzodiazepine for most of the year, clonazolam detection increased towards the end of the year and even exceeded that of bromazolam in November and December. Despite this, bromazolam remained the most prevalent designer benzodiazepine in 2024 based on annual positivity.

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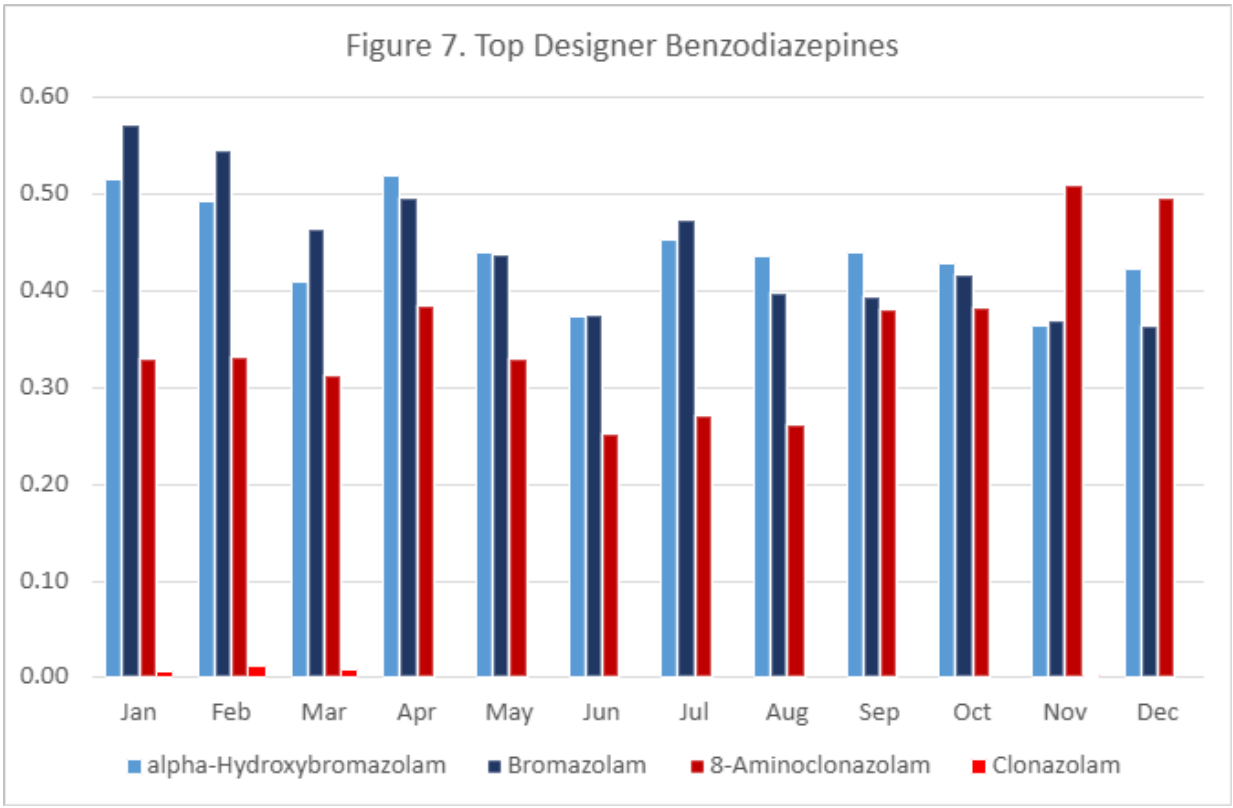

The positivity of other designer benzodiazepines detected in 2024 is shown in **Figure 8**. Designer benzodiazepines with less than 10 detections in the year were not included in the graph. After bromazolam and clonazolam, the next most prevalent designer benzodiazepine in 2024 was etizolam which showed an increase in detection midyear but ended the year with a similar positivity to what it started the year with. The flubromazepam metabolite, 3-OH flubromazepam, had an annual positivity approximately half that of etizolam and was the next most prevalent designer benzodiazepine in 2024. Flualprazolam positivity was greatest in Q1 and Q4, but its midyear positivity was often not even half that. N-desmethyl Adinazolam detection fluctuated but it was detected in every month whereas desalkylgidazepam detection was sporadic, mostly being detected in Q4. Pyrazolam was added to Aegis testing in April and was detected for the remainder of the year but at a somewhat low positivity.

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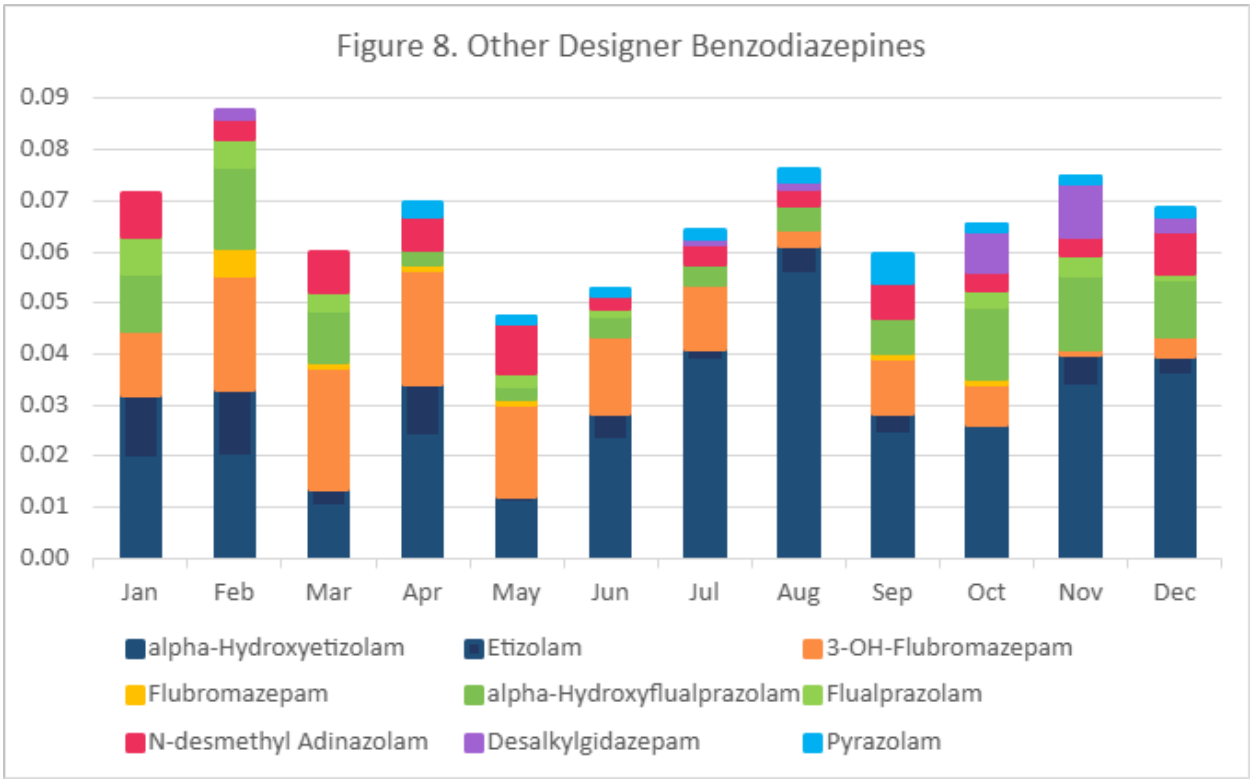

SYNTHETIC CANNABINOIDS

Synthetic cannabinoids and synthetic stimulants, specifically cathinones, were among the first classes of NPS available in the United States with synthetic cannabinoids appearing first and in greater abundance than synthetic stimulants. However, reports of detection of these two classes of NPS have been declining in recent years, likely due to legislation that targets specific chemical structures and entire classes of substances. In 2021, China issued a class-wide ban of a specific structural class of synthetic cannabinoids. The prevalence of the top synthetic cannabinoids detected in 2024 are shown in **Figure 9**. MDMB-4en-PINACA, mainly via detection of its butanoic acid metabolite, has been the most predominant synthetic cannabinoid detected at Aegis since at least 2022 and remained so in 2024. In April of 2023, The Federal Register issued a notice of intent to temporarily place MDMB-4en-PINACA in schedule I of the CSA.[16] However, this has not yet seemed to impact its detection as its positivity was greatest in Q4 of 2024. MDMB-INACA is a synthetic cannabinoid precursor that can be used to make several different synthetic cannabinoids including MDMB-4en PINACA.[17] It was added to Aegis' synthetic cannabinoid testing in April and became the second most prevalent synthetic cannabinoid detected in 2024. It was often detected with MDMB-4en-PINACA.

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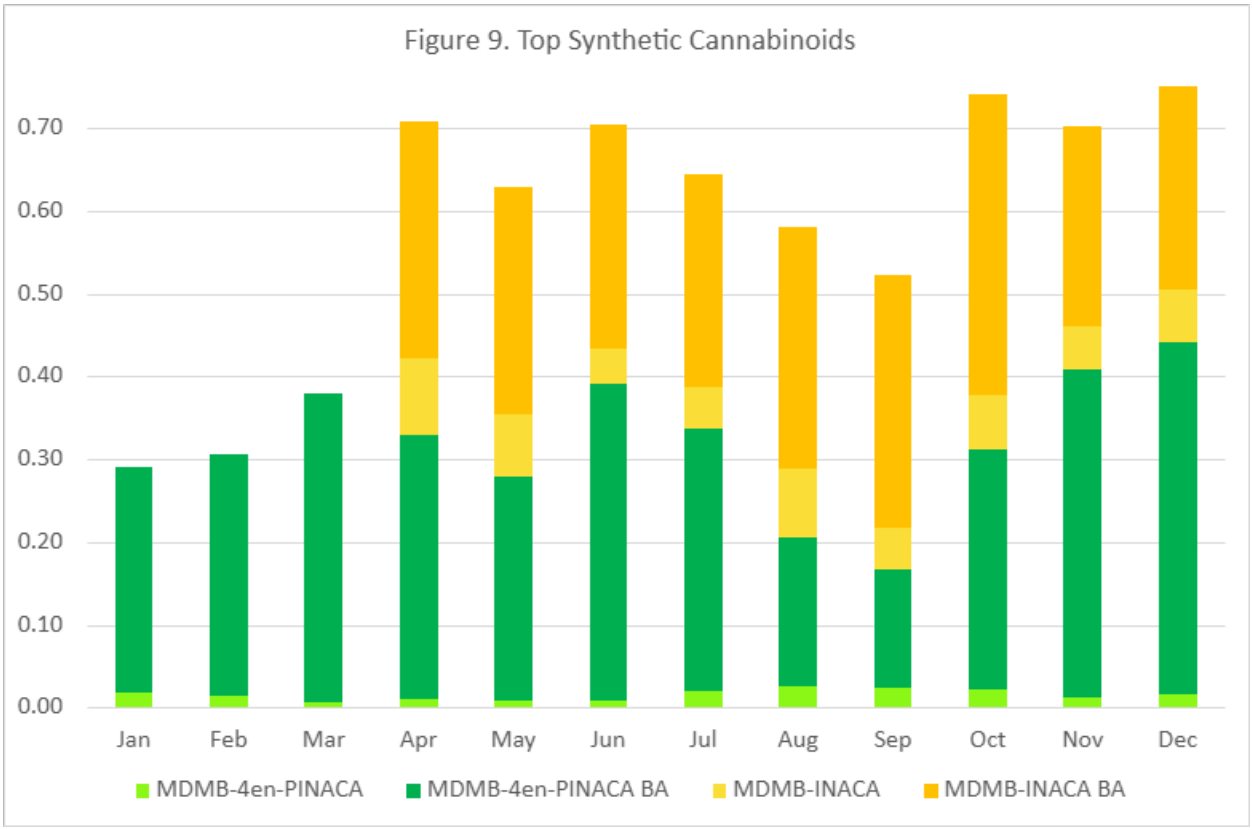

The prevalence of other synthetic cannabinoids detected in 2024 is shown in **Figure 10**. Synthetic cannabinoids with less than 10 detections in the year were not included in the graph unless they are related to a compound (e.g. parent compound) with greater positivity. 4F-MDMB-BUTINACA and its metabolites represent the third most prevalent synthetic cannabinoid detected in 2024, with the N-BA metabolite accounting for the majority of the positivity. Detection of 4F-MDMB-BUTINACA N-BA metabolite was greatest in Q1, but its overall positivity at the end of the year was decreased compared to the first of the year. 5F-MDMB-PINACA and its metabolites were the next most prevalent synthetic cannabinoid, mainly via detection of the M7 metabolite which increased significantly in Q4, with its average positivity in Q4 increasing approximately 1400% over the average positivity of Q1 through Q3. ADB-INACA and MDMB-BUTINACA and its BA metabolite were added to Aegis' testing in April and despite only being tested for in 3 of 4 quarters of the year, these synthetic cannabinoids were the next most prevalent synthetic cannabinoids with ADB-INACA having slightly greater positivity. ADB-BUTINACA and its metabolite were the third most prevalent synthetic cannabinoid detected in 2023, however in 2024 its relative prevalence decreased and its average detection in the second half of the year decreased to only approximately 20% of its average detection in the first half of the year. 4-CN-CUMYL-BUTINACA Metabolite M10 detection was mostly only detected in the second half of 2024. MMB-4en-PINACA BA metabolite was sporadically detected but mostly in Q1. ADB-PINACA PA metabolite and 5F-MDMB-PICA M7 metabolite were minimally and sporadically detected thought the year.

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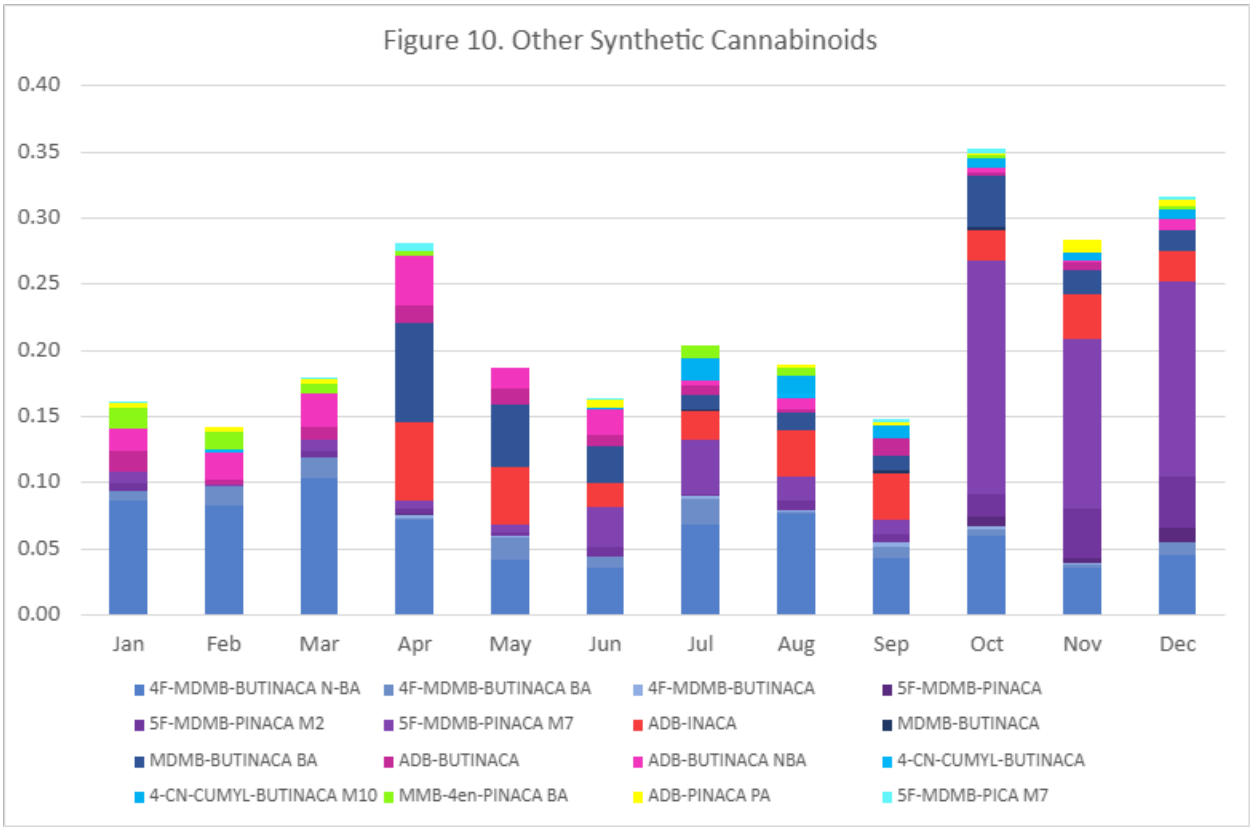

SYNTHETIC STIMULANTS

Synthetic stimulants tested at Aegis include analogs of amphetamine and methylphenidate as well as cathinones, which have been erroneously sold as "bath salts". The prevalence of the synthetic stimulants in 2024 is shown in **Figure 11**. Synthetic stimulants with less than 10 detections in the year were not included in the graph. In 2022 pentylone replaced eutylone as the most prevalent synthetic stimulant detected. Since pentylone was added to Schedule I of the CSA in 2017, the increase in its detection in 2022 was suspected to be due to it being a metabolite of the novel synthetic stimulant N,N-dimethylpentylone.[18,19] N,N-dimethylpentylone was added to Aegis Synthetic Stimulant testing in August of 2022 and since then N,N-dimethylpentylone and metabolite pentylone have been the most predominant synthetic stimulants detected at Aegis and this continued in 2024. Uncharacteristically, their positivity declined significantly throughout the year and N,N-dimethylpentylone and pentylone positivity in December was only approximately 15% and 7% respectively of that in January. The order of the top most prevalent synthetic stimulants did not change from 2023. Eutylone was again the second most prevalent synthetic stimulant detected with greater positivity in the second half of the year compared to the first. N-cyclohexyl Methylone was the third most prevalent and increased in positivity in the first half of the year but then dropped significantly in the second half and increased slightly to end the year at roughly the same positivity it started with. Other synthetic stimulants had low and somewhat sporadic detection.

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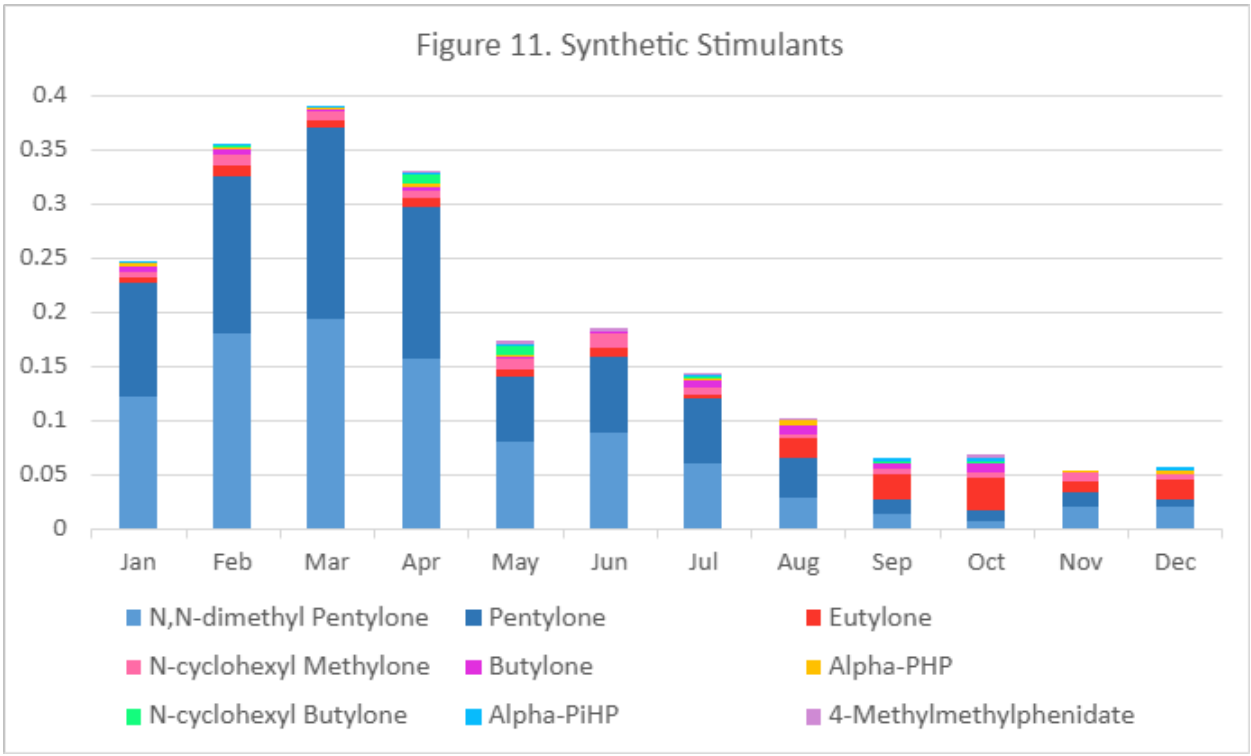

The prevalence of hallucinogens/dissociatives in 2024 are shown in Figure 12. 2F-Deschloroketamine and its metabolite 2F-deschloronorketamine first appeared in April of 2022. 2F-deschloronorketamine became the most prevalent hallucinogen/dissociative compound detected in 2022 and this continued in both 2023 and 2024. 2F-2-oxo-PCE is structurally similar to ketamine but is not currently a scheduled drug. It was added to Aegis' NPS testing in September of 2023 and was so frequently detected in the fourth quarter that it became the third most frequently detected hallucinogen/dissociative compound. In May of 2024, CFSRE released a public alert warning that 2F-2-oxo-PCE has been detected in recreational drug markets across North America.[20] 2F-2-oxo-PCE was the second most prevalent hallucinogen/dissociative compound in 2024. After reaching maximum positivity in May, 2F-2-oxo-PCE precipitously decreased the remainder of the year. The third most prevalent hallucinogen/dissociative compound in 2024 was the PCP analog, 3-OH-PCP which was newly detected in the second half of 2022 and was the second most frequently detected hallucinogen/dissociative in 2023. Its detection in 2024 was variable but showed an overall decrease in positivity. Other hallucinogen/dissociative compounds had low and somewhat sporadic detection.

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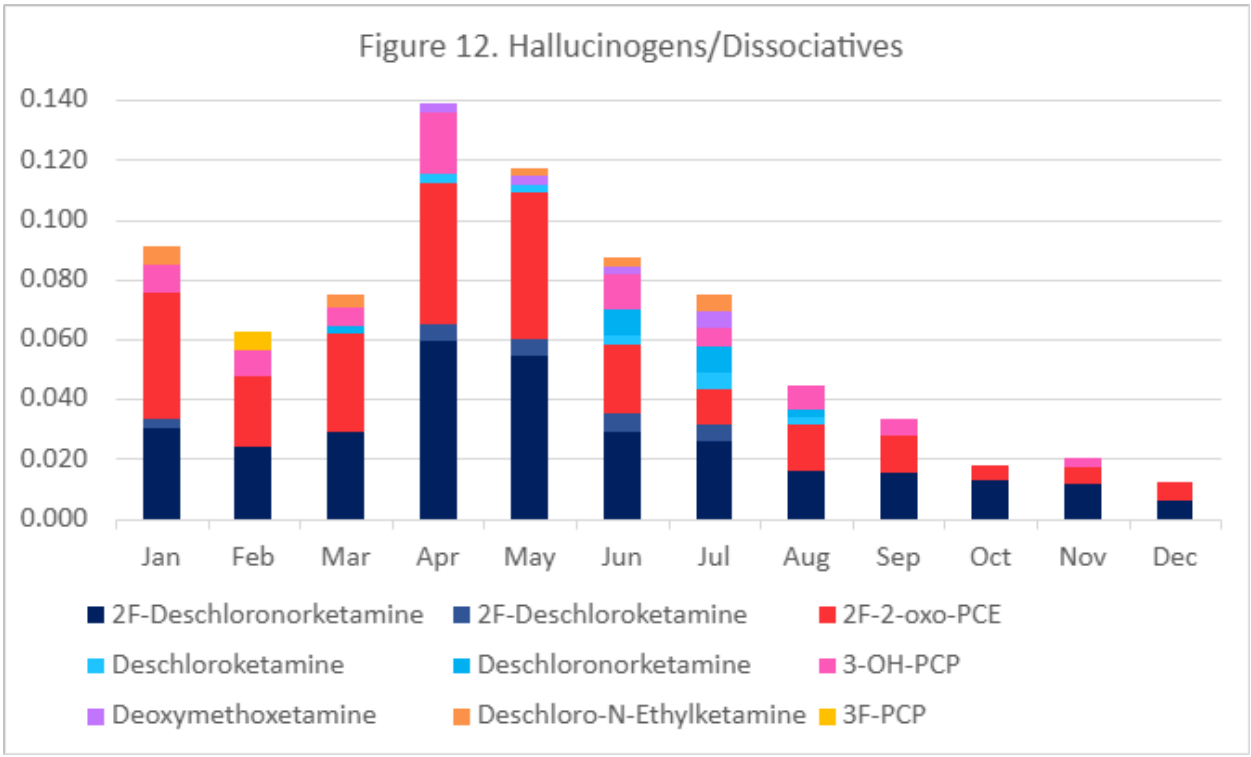

ALL NPS

The top NPS detected at Aegis in 2024 irrespective of classification are shown in **Table 1**.

Table 1. Top NPS Detected at Aegis in 2024	**NPS Classification**
Xylazine/4-hydroxy Xylazine	NPS - Other
Fluoro Fentanyl/Despropionyl FF/Fluoro Norfentanyl	Designer Opioids
Bromazolam/alpha-hydroxybromazolam	Designer Benzodiazepines
8-Aminoclonazolam	Designer Benzodiazepines
N-desethyl Metonitazene/Metonitazene	Designer Opioids
4-hydroxy Nitazene	Designer Opioids
MDMB-4en-PINACA BA	Synthetic Cannabinoids
MDMB-INACA BA	Synthetic Cannabinoids
Medetomidine/3-hydroxy medetomidine	NPS - Other
N-Pyrrolidino 4-OH Nitazene	Designer Opioids
Valeryl Fentanyl Carboxy Metabolite	Designer Opioids
Tianeptine MC5/Tianeptine	NPS - Other
Phenibut	NPS - Other
Norcarfentanil	Designer Opioids
N,N-dimethyl Pentylone/Pentylone	Synthetic Stimulants

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NOTICE: The information above is intended as a resource for health care providers. Providers should use their independent medical judgment based on the clinical needs of the patient when making determinations of who to test, what medications to test, testing frequency, and the type of testing to conduct. (Calibri 10pt)

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