Drugs of Abuse and Out of Scope Findings

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IACME, Iowa Association of County Medical Examiners
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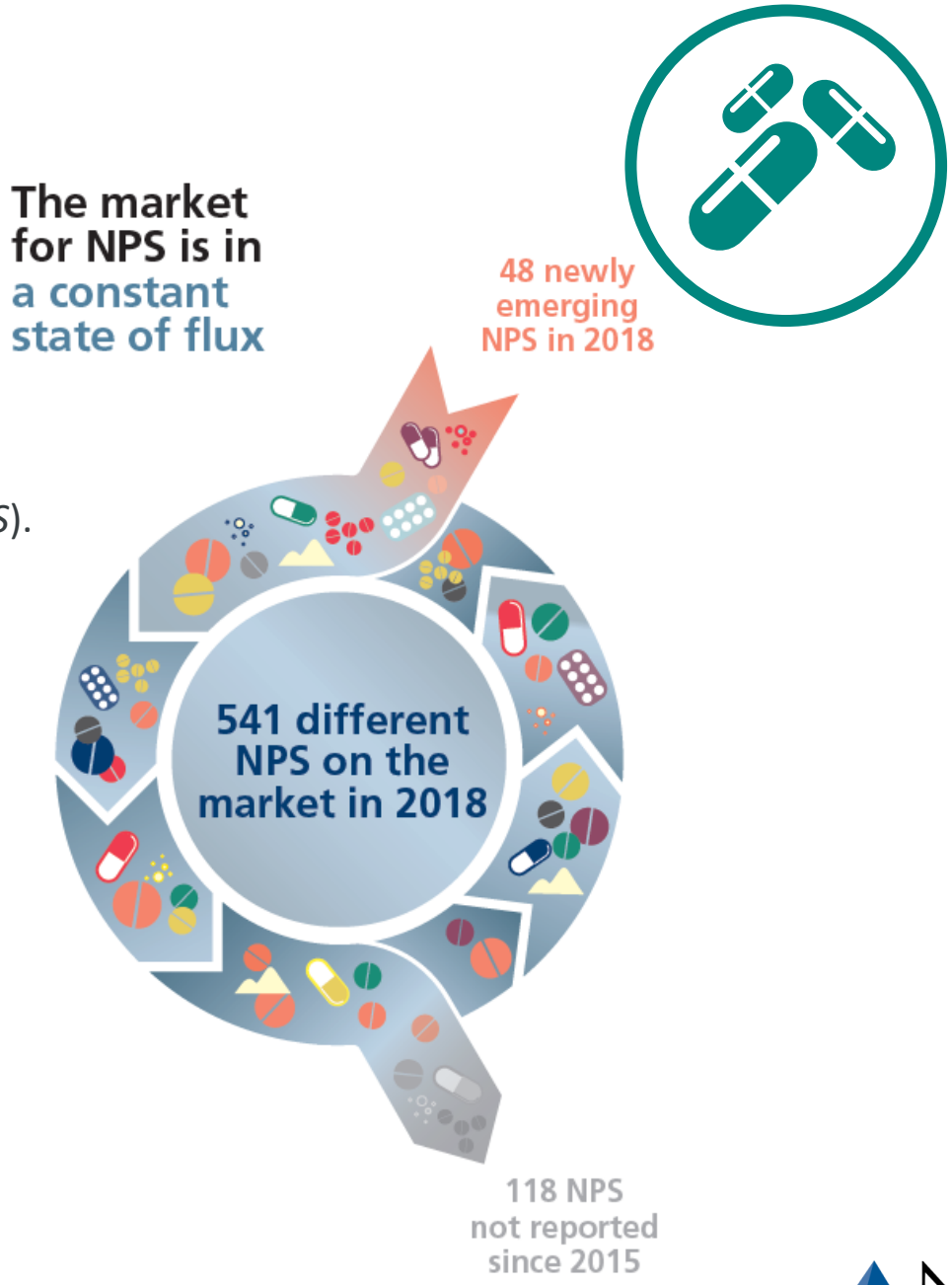
Disclosure

- Carolina Noble is a Forensic Toxicologist at NMS Labs, a commercial provider of toxicology and other forensic testing services.
- The opinions presented do not necessarily represent those of NMS Labs or any of my NMS Labs colleagues.

Novel Psychoactive Substances

Novel Psychoactive Substances (NPS): substances of abuse which are emerging on the drug scene without being controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychoactive Substances (UNODC, *https://www.unodc.org/LSS/Page/NPS*).

NPS have been steadily emerging on the illicit drug scene since 2008. Illicit manufacturing of different classes of NPS and the alarming increase of NPS-related overdoses over the last decade, have raised the need of investing efforts to constantly **monitor** their appearance, **elucidate** pharmacological aspects and **implement** drug control policies. Changes in trends in NPS are difficult to **predict**, and they usually are subject to demographic, economic and social aspects.



World Drug Report 2020 (United Nations publication, Sales No. E.20.XI.6)

Q2 2022 DEA Tox data

Figure 2A. Number of Substances Identified for Each NPS Class

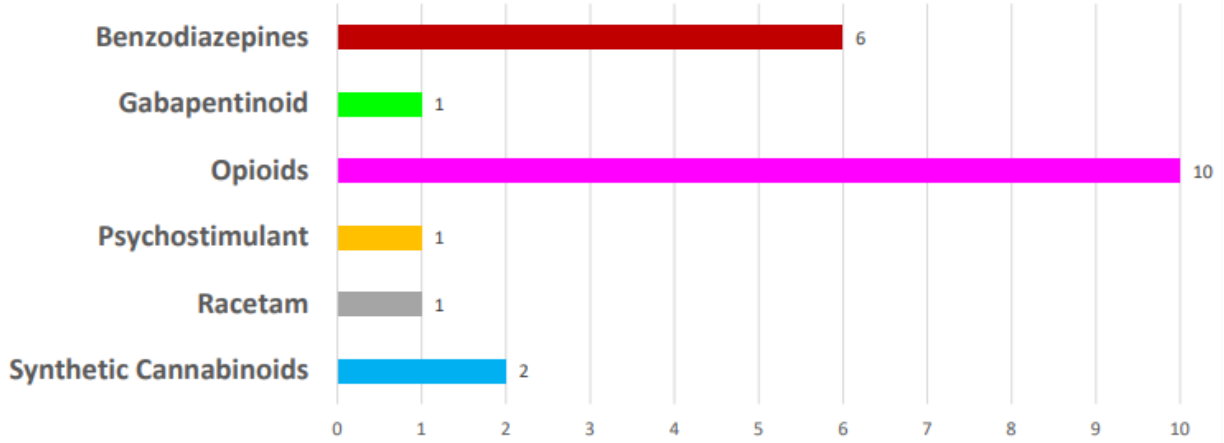
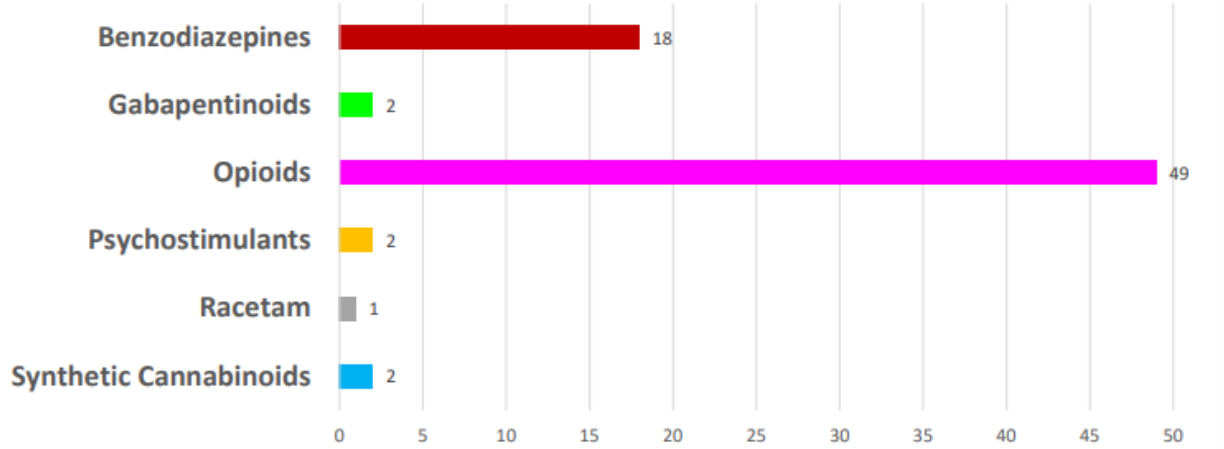
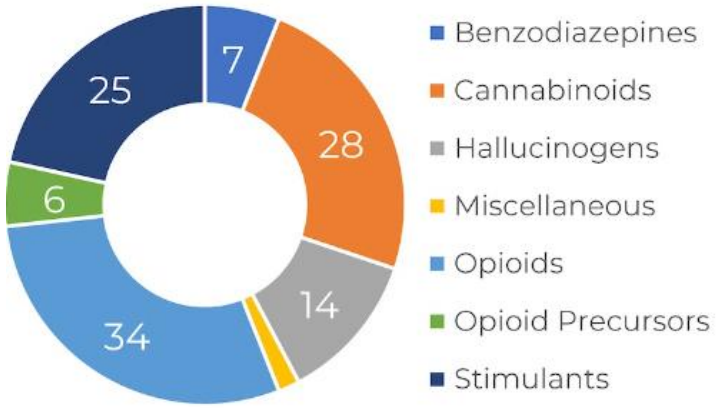


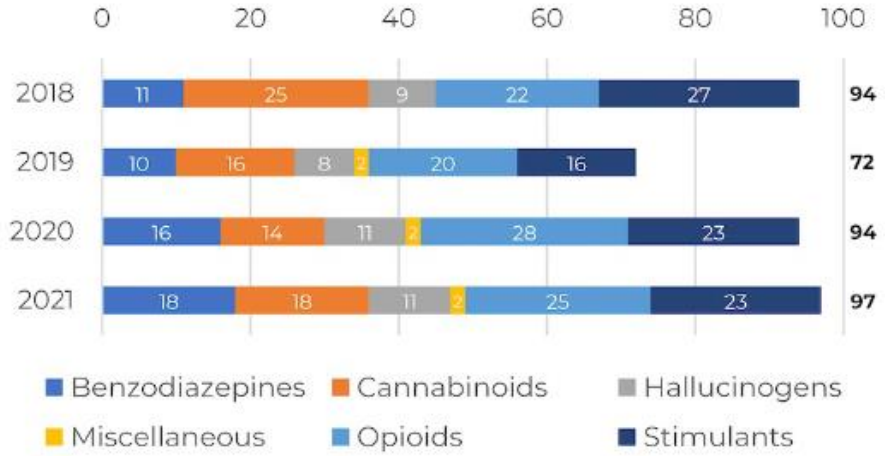
Figure 2B. Total Encounters for Each NPS Class



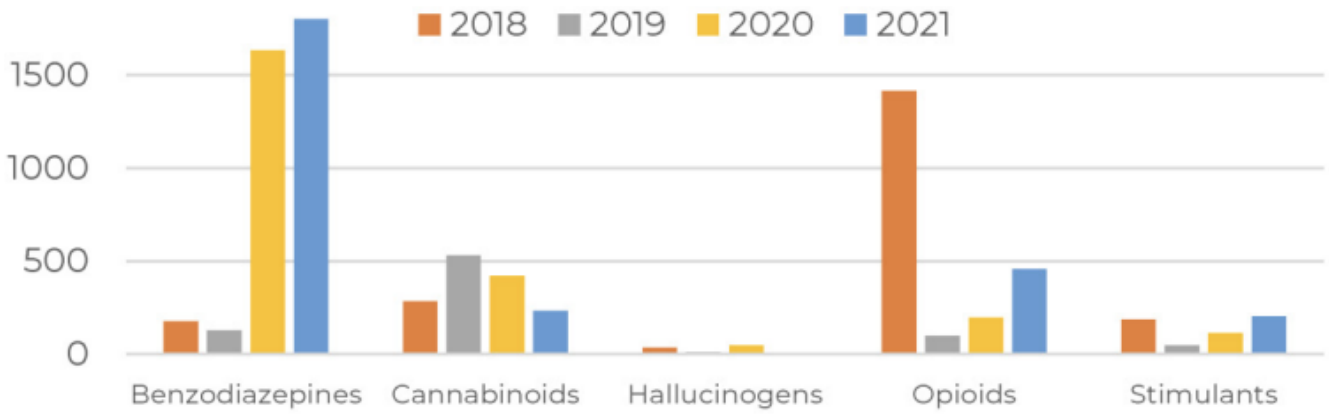
Source: https://www.deadiversion.usdoj.gov/dea_tox/quarterly_reports/2nd_Quarter_2022_DEA_TOX_08162022.pdf



Breakdown, by subclass, of newly discovered NPS, 2018-2021



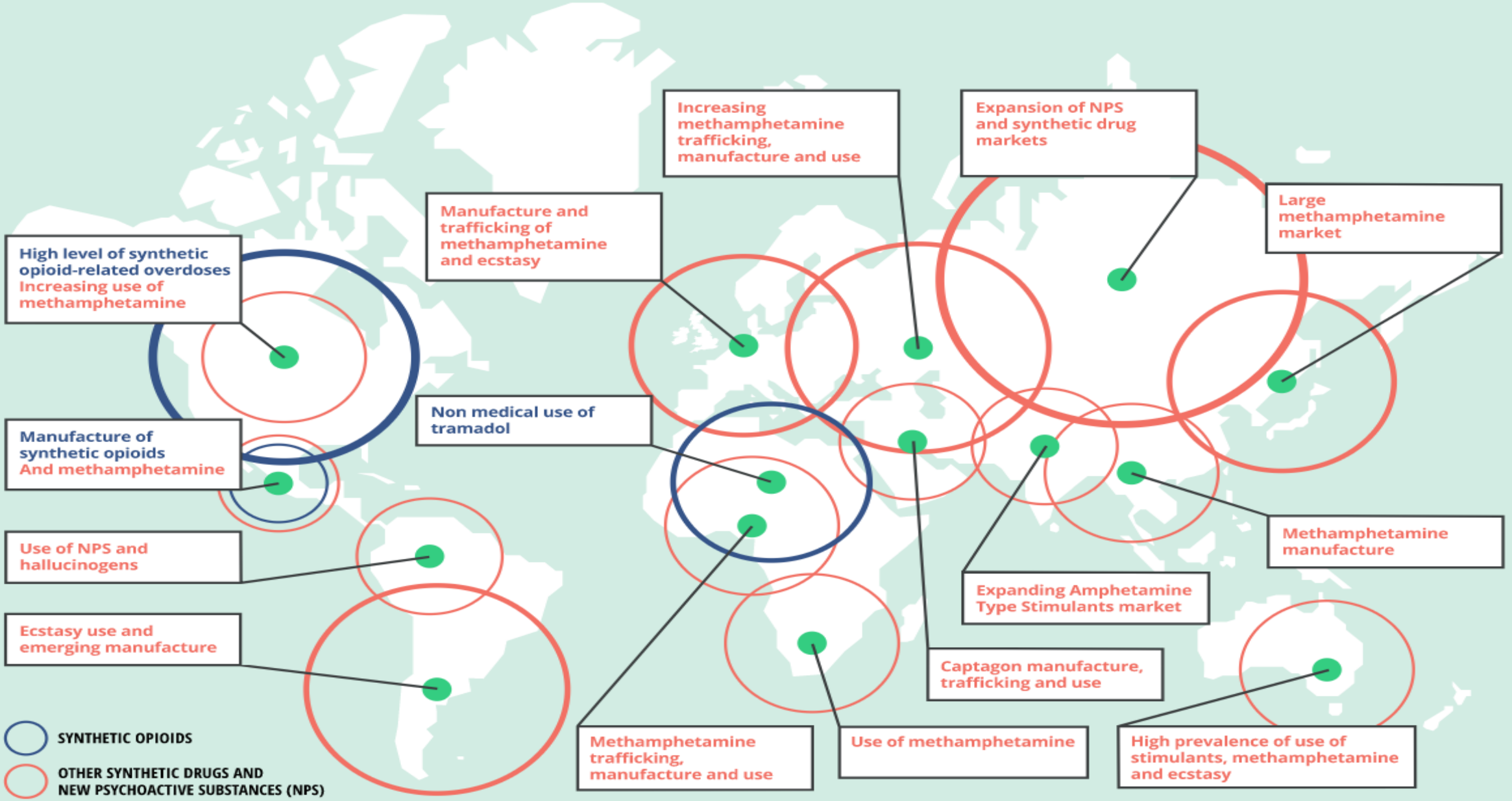
Individual NPS detected each year, cumulative since 2018.



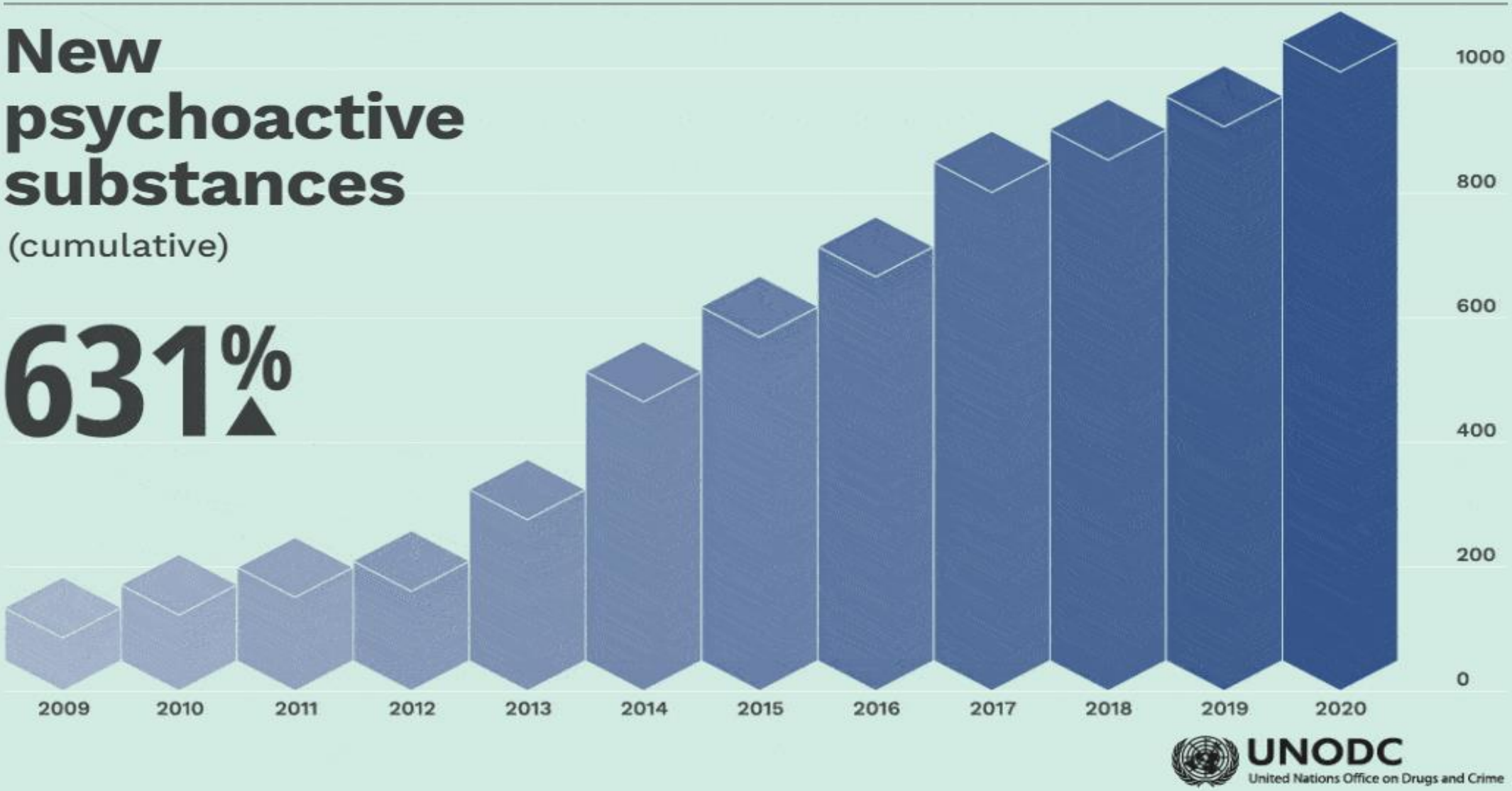
Total number of NPS detections among all samples analyzed since 2018.

Source: https://www.cfsre.org/nps-discovery/trend-reports

Source: https://syntheticdrugs.unodc.org/syntheticdrugs/en/strategy.html



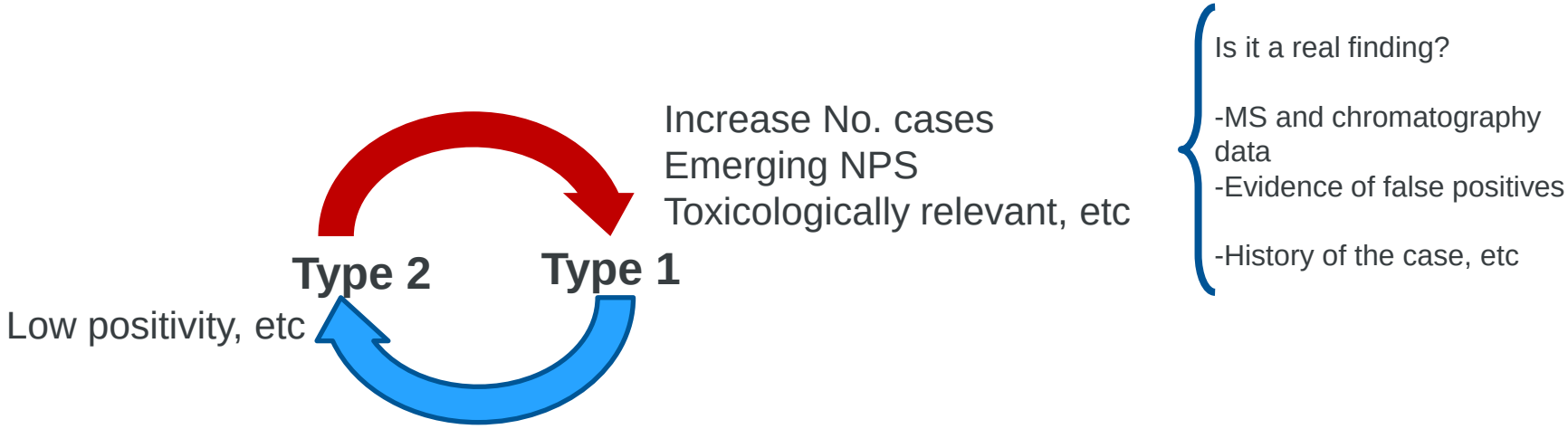
Source: https://syntheticdrugs.unodc.org/syntheticdrugs/en/strategy.html



Out-of-Scope Findings

Out-of-Scope findings are classified into **Type 1** and **Type 2**.

Type 1 analytes are OOS findings that require, if it does not have it already, a quali/quanti confirmation method after its detection by screening methods. Detection of T1 analytes are evaluated by a toxicologist for further actions



Workflow (1)

Client submit samples for expanded panel e.g.: 8052X (X: S/P, B, U)

Liquid-liquid extraction (1-Chlorobutane:Ethyl acetate, 70:30)

LC-TOF Agilent Jet Stream 6230

Data processing
-Library~ 265 compounds (in- scope compounds)
-OOS library

OOS found?

Yes

No

No action

Screening Dept places ADDONFSC

TOX queue

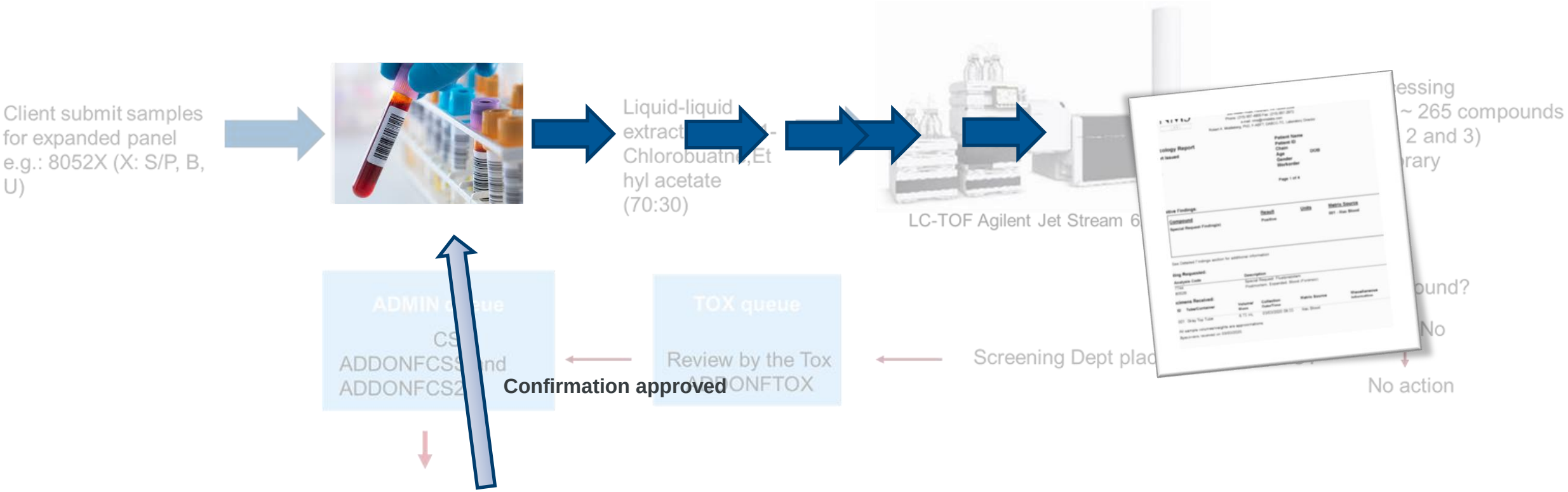
Review by the Tox
ADDONFTOX

ADMIN queue

CS
ADDONFCSS and
ADDONFCS2

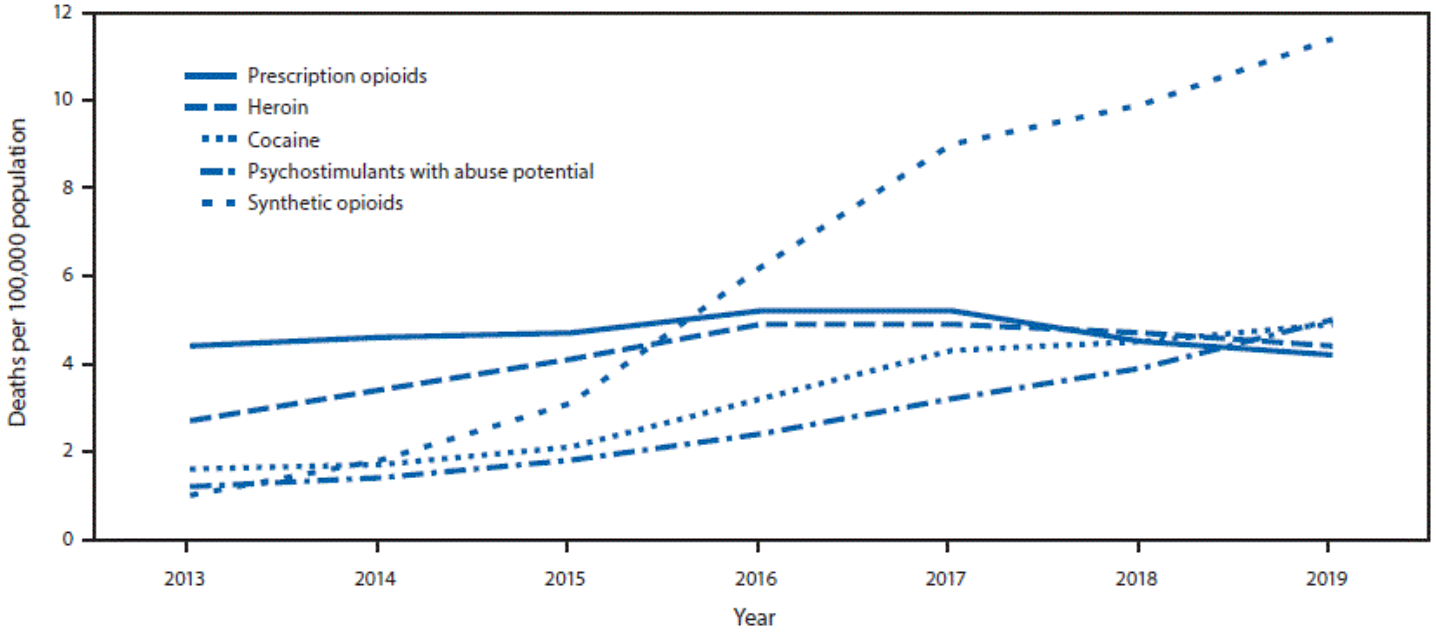
Client is informed about the finding(s) and approves or not confirmation

Workflow (2)


Client submit samples for expanded panel e.g.: 8052X (X: S/P, B, U)
Liquid-liquid extract
Chlorobuatn
hyl acetate (70:30)
LC-TOF Agilent Jet Stream 6
essing
~ 265 compounds
2 and 3)
rary
ound?
No
No action
Screening Dept plac
TOX queue
Review by the Tox
ADDONFTOX
ADMIN
ADDONFCS
Confirmation approved
Client is informed about the finding(s) and approves or not OOS confirmation

Synthetic opioids

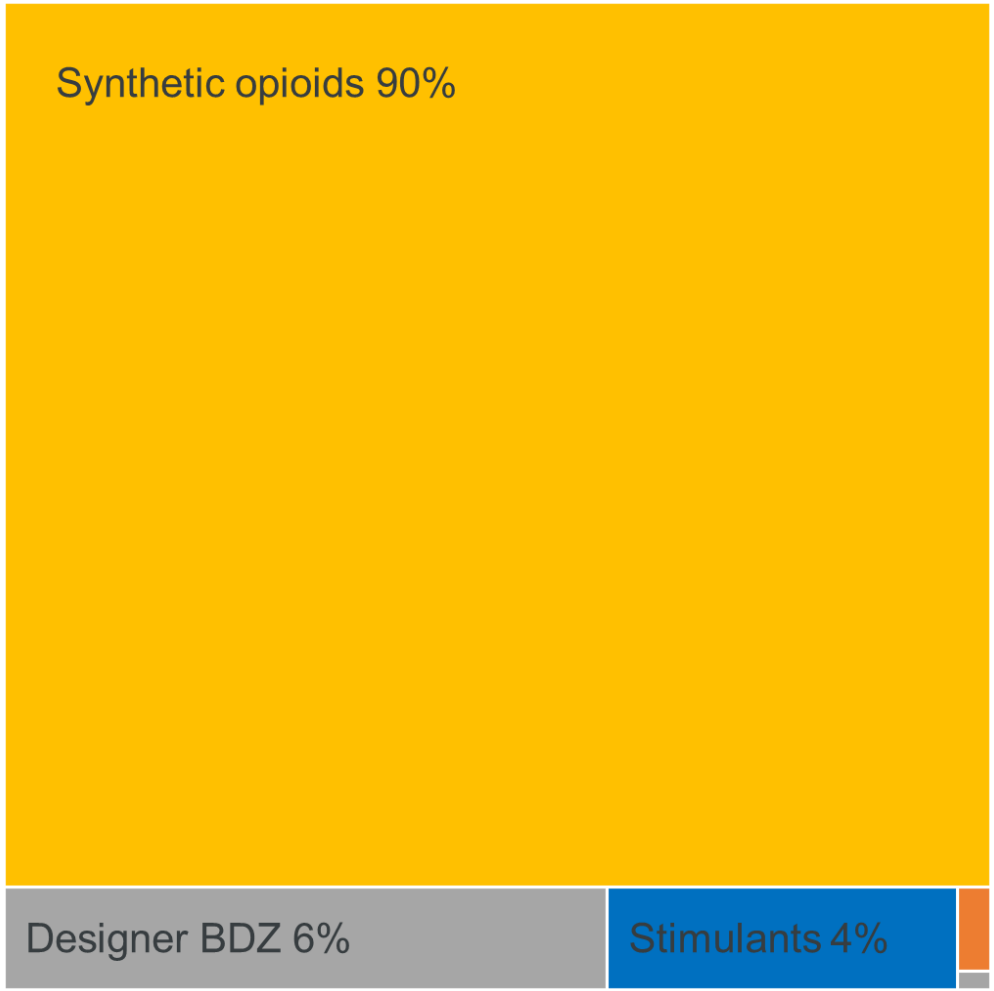
FIGURE 1. Age-adjusted rates* of drug overdose deaths† involving prescription opioids,§ heroin,¶ cocaine,** psychostimulants with abuse potential,†† and synthetic opioids other than methadone§§,¶¶ — United States, 2013–2019

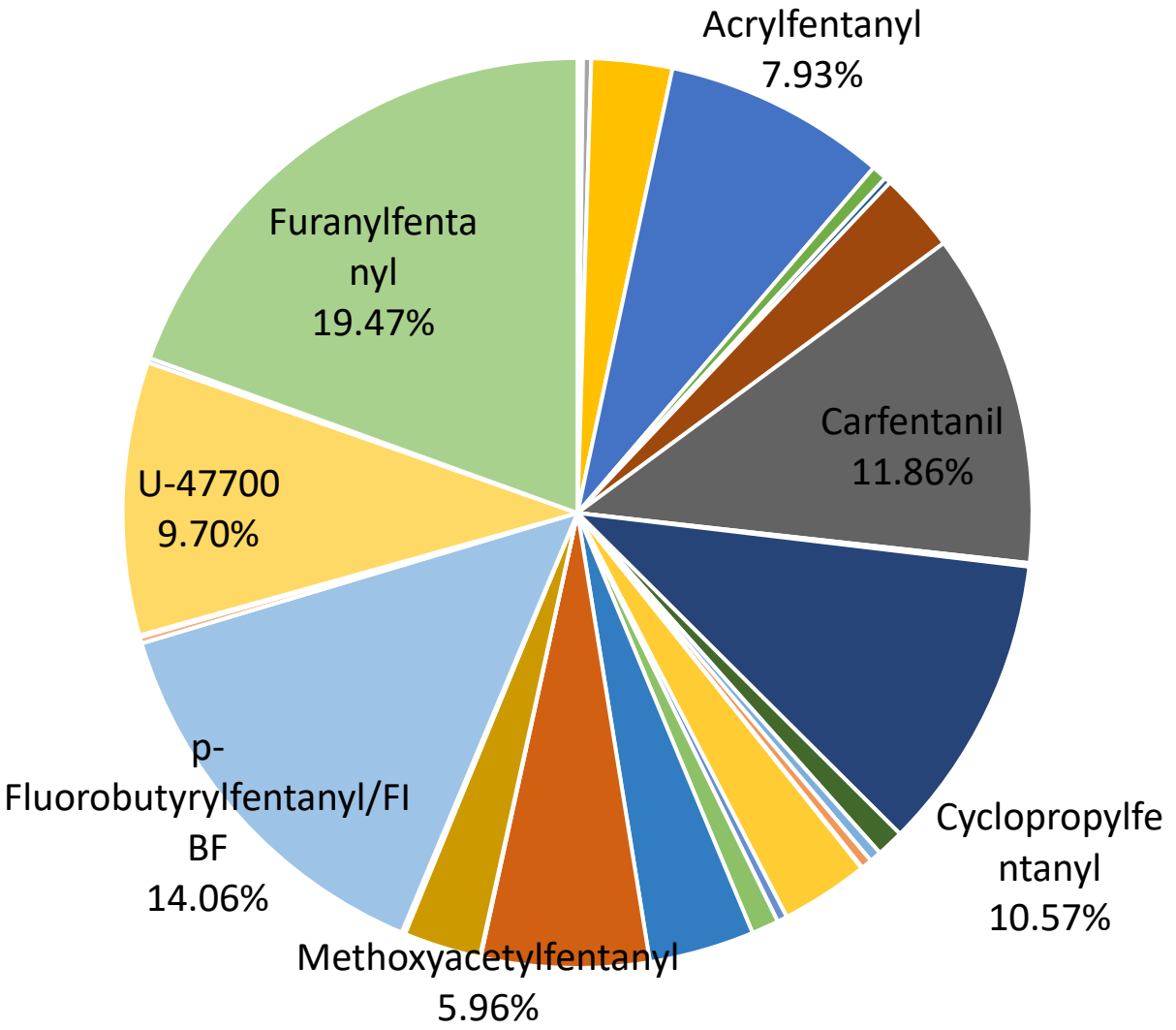


Source: National Vital Statistics System, Mortality File. https://wonder.cdc.gov/

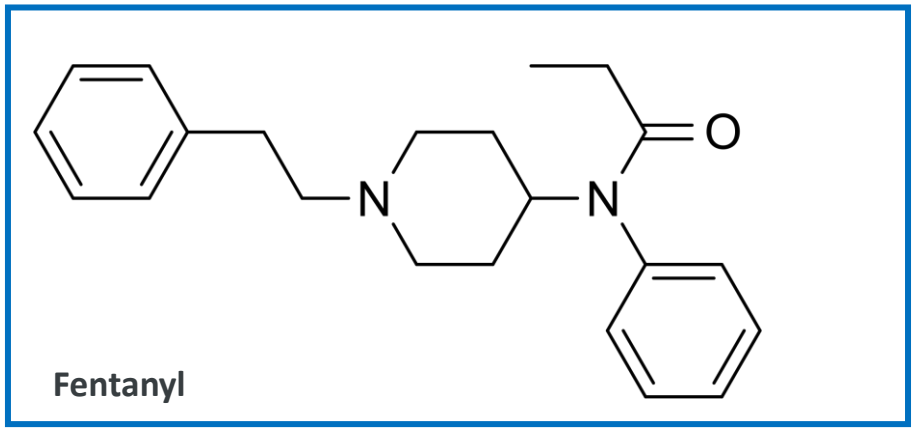
https://www.cdc.gov/mmwr/volumes/70/wr/mm7006a4.htm?s_cid=mm7006a4_w

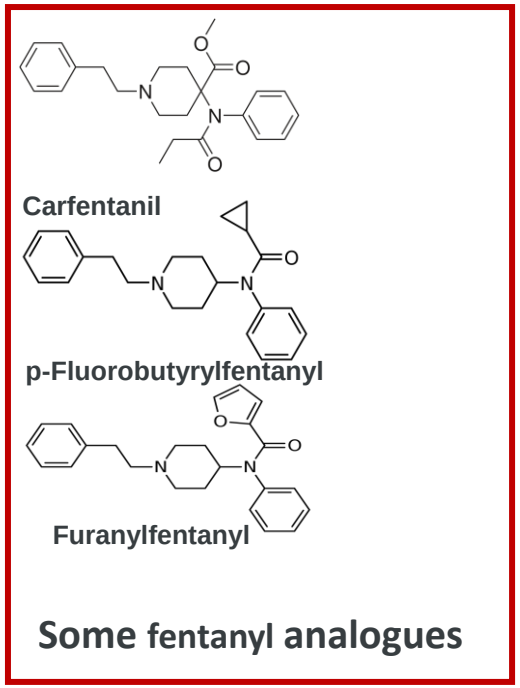
NPS confirmed in 2017

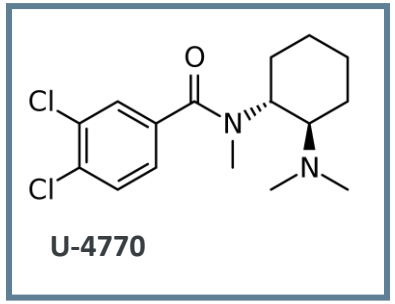


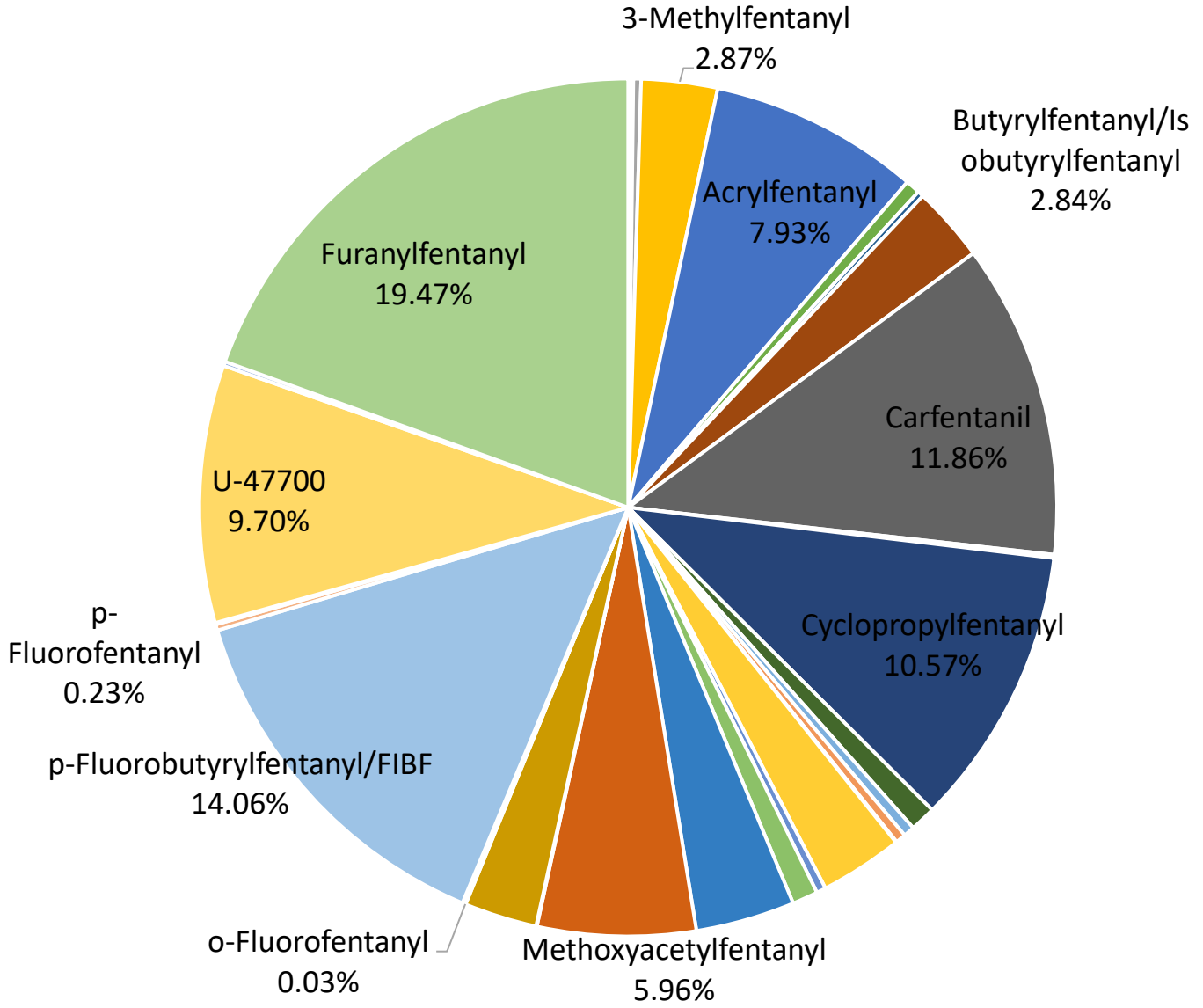


NPS confirmed in 2017

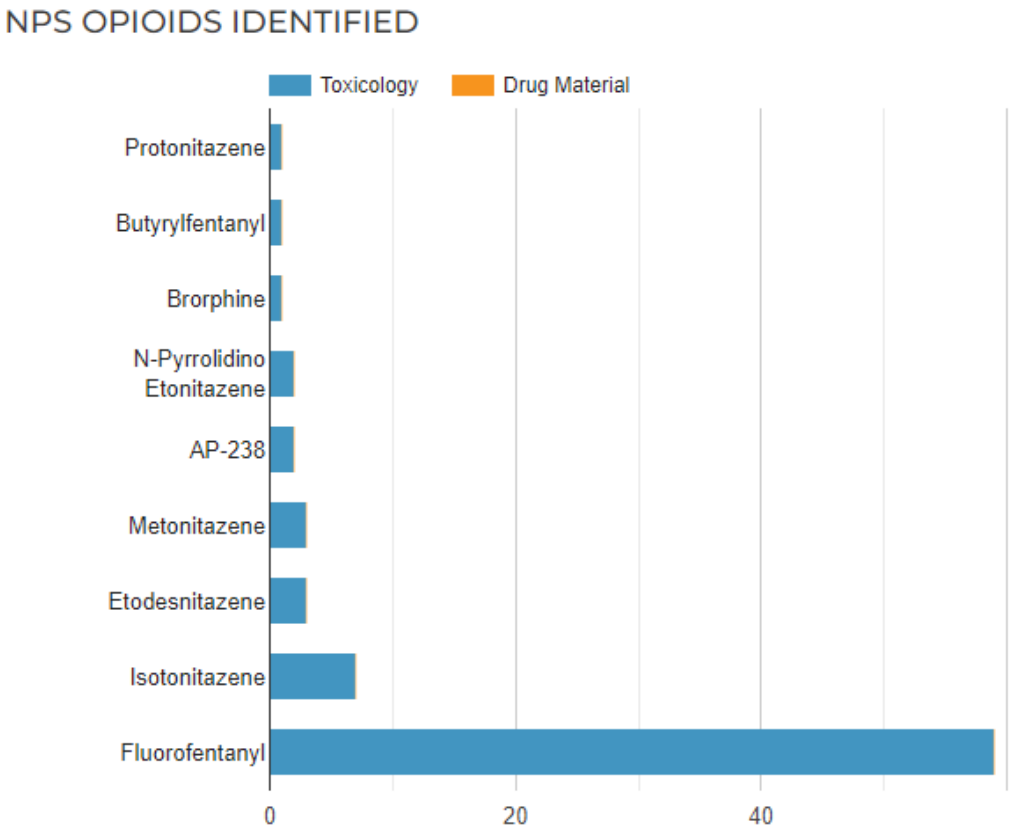




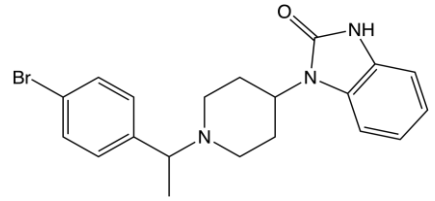




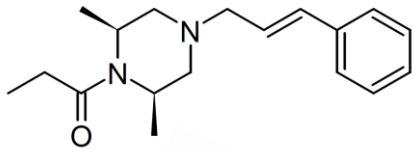
Synthetic opioids in 2022



https://www.cfsre.org/nps-discovery/trend-reports/nps-opioids/report/49?trend_type_id=2



Brorphine



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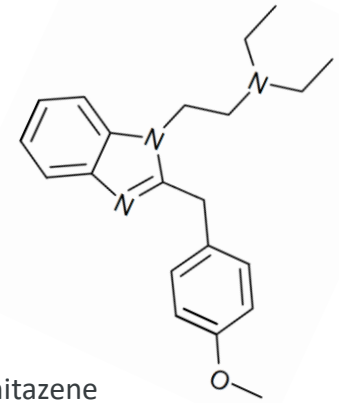
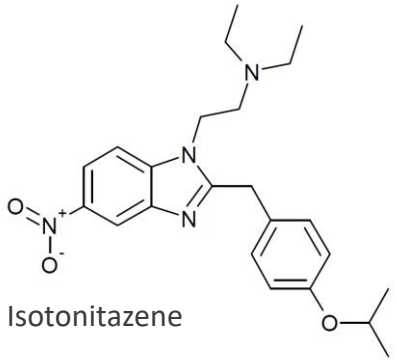
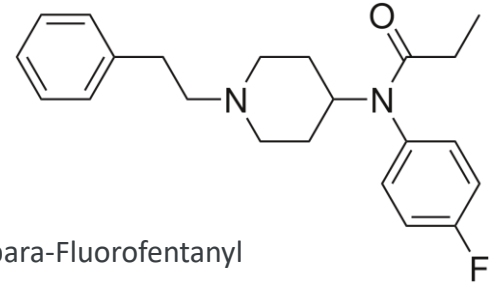


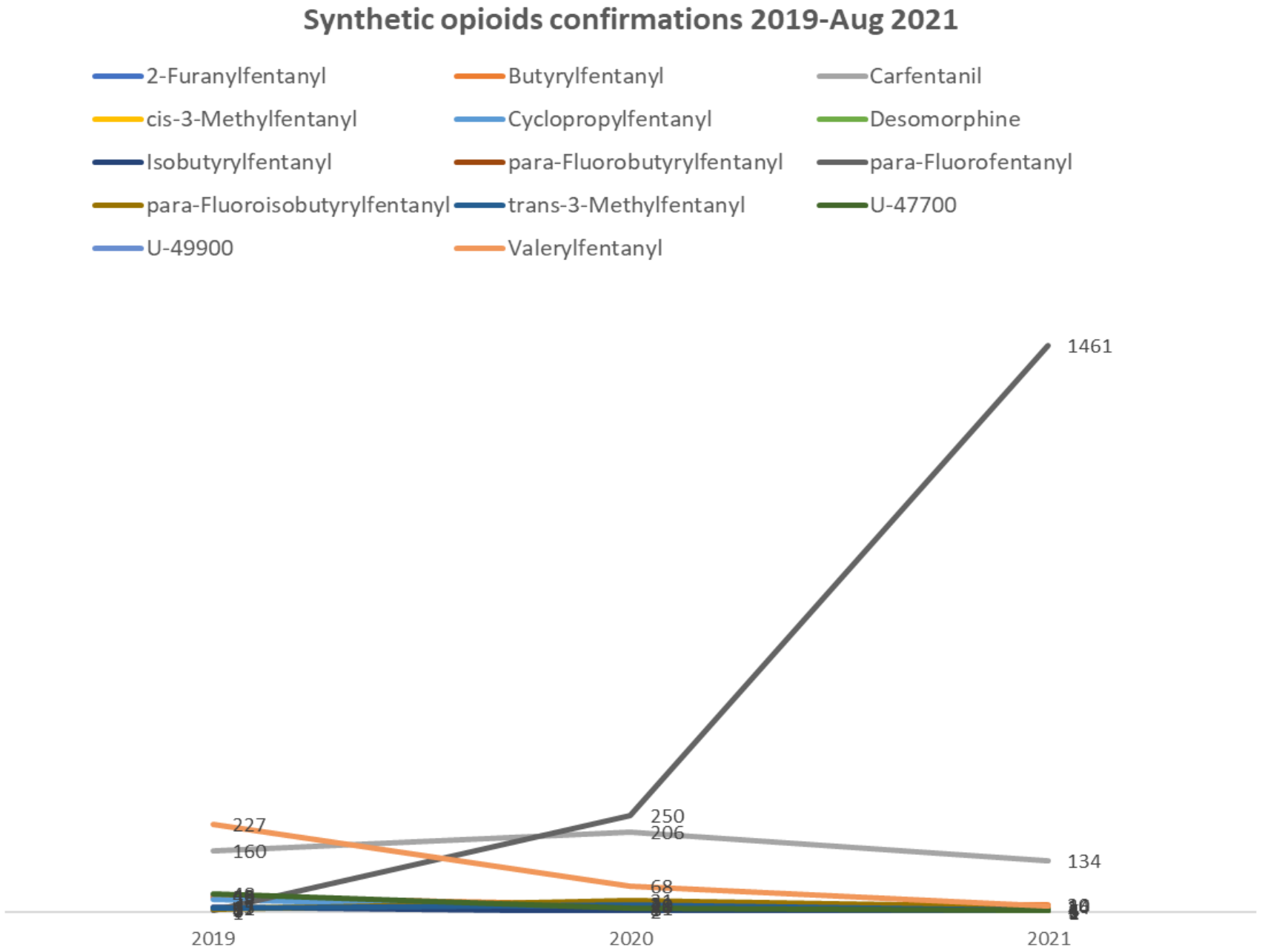
Metonitazene

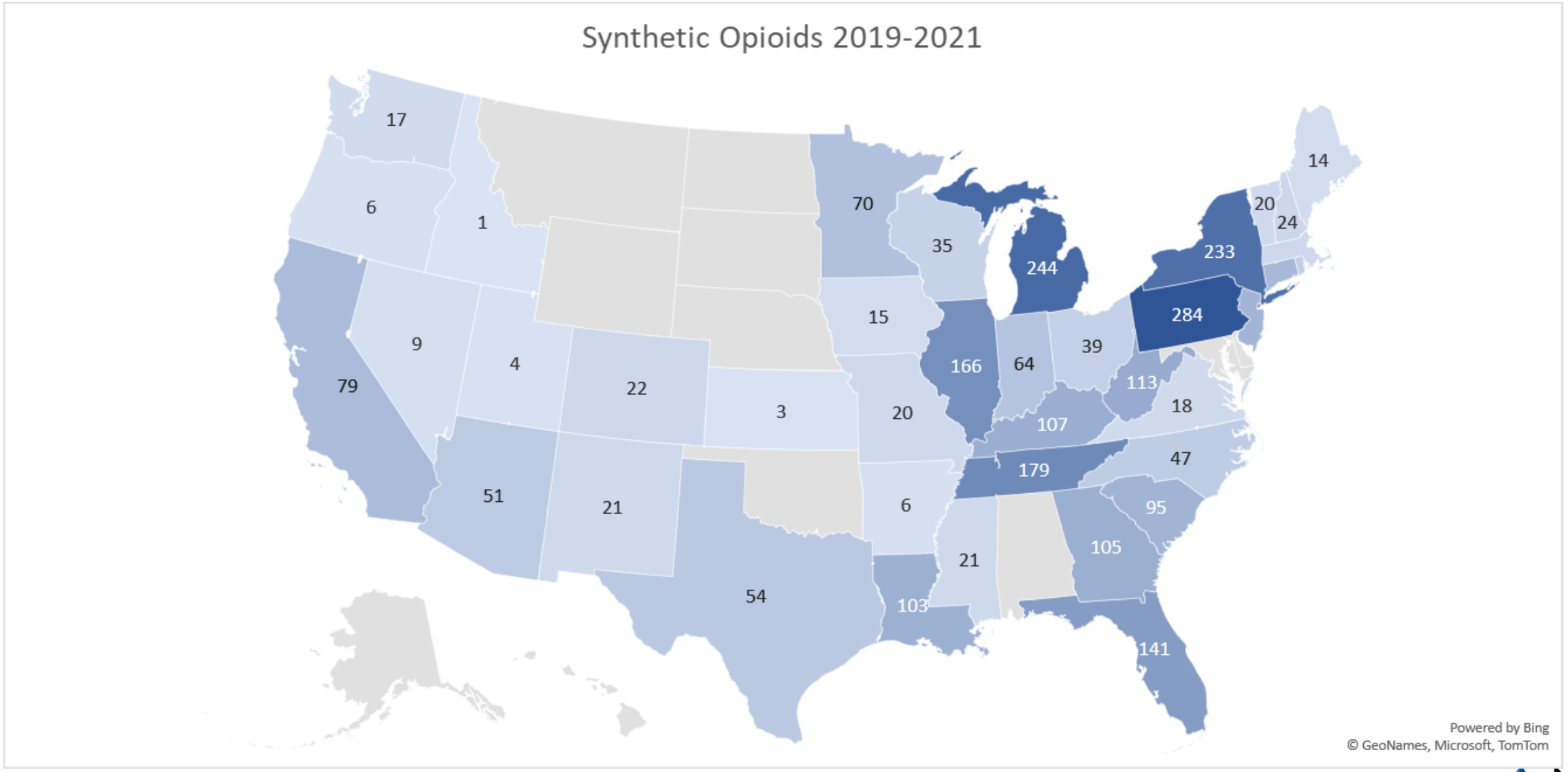


Isotonitazene



para-Fluorofentanyl


Synthetic opioids confirmations 2019-Aug 2021
2-Furanylfentanyl
Butyrylfentanyl
Carfentanil
cis-3-Methylfentanyl
Cyclopropylfentanyl
Desomorphine
Isobutyrylfentanyl
para-Fluorobutyrylfentanyl
para-Fluorofentanyl
para-Fluoroisobutyrylfentanyl
trans-3-Methylfentanyl
U-47700
U-49900
Valerylfentanyl
1461
227
250
206
160
134
68
2019
2020
2021


Synthetic Opioids 2019-2021
17
6
1
70
35
14
20
24
244
233
284
15
9
4
22
3
20
166
64
39
113
18
107
79
179
47
51
21
6
95
105
21
54
103
141
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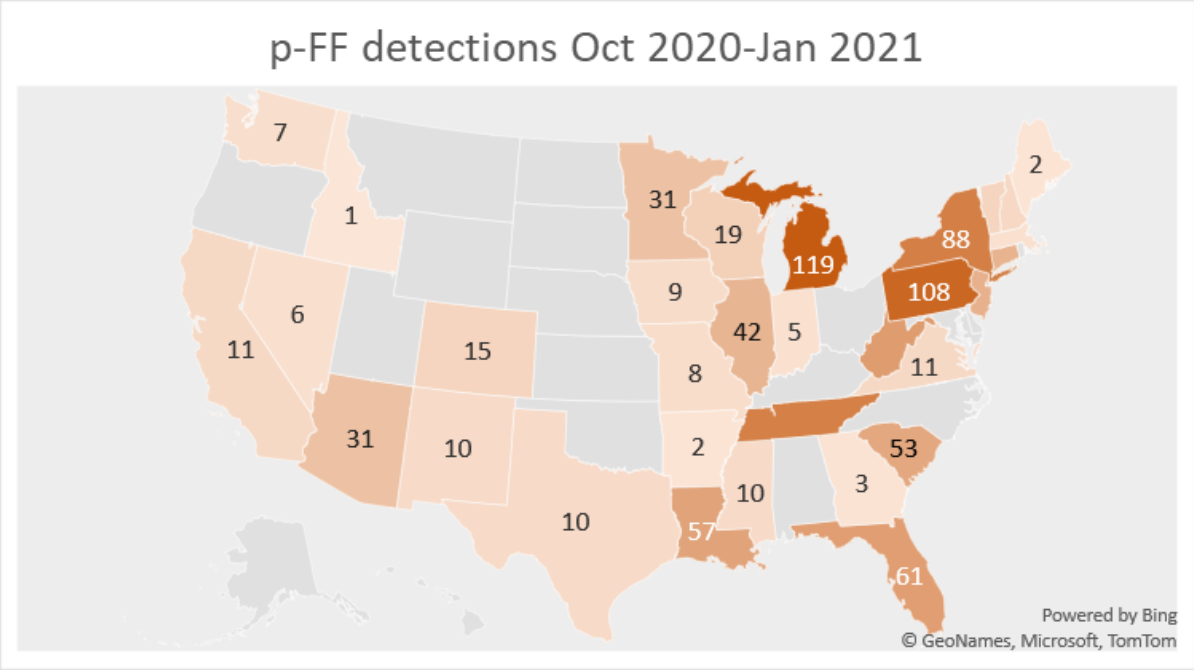
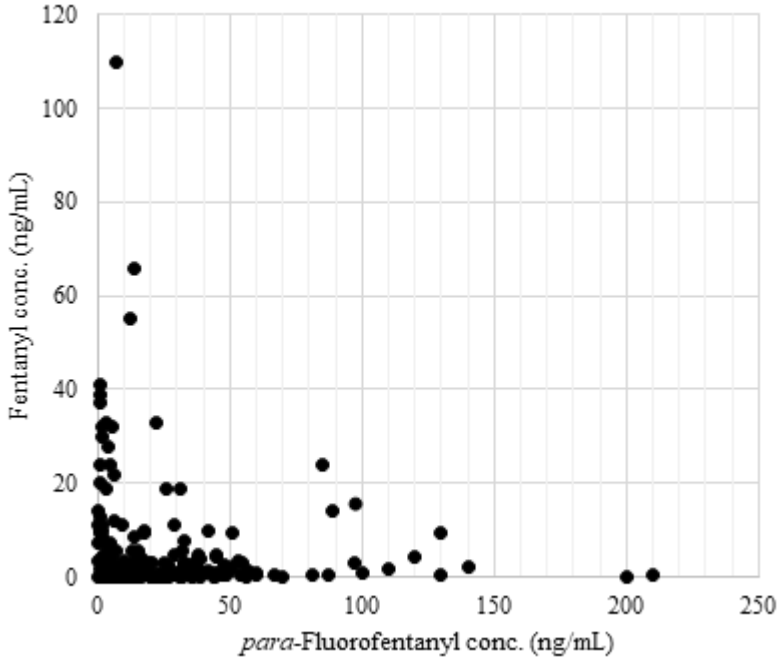
The role of para-Fluorofentanyl in forensic casework

In Q3 2020, para-Fluorofentanyl (pFF) identifications in blood samples increased significantly

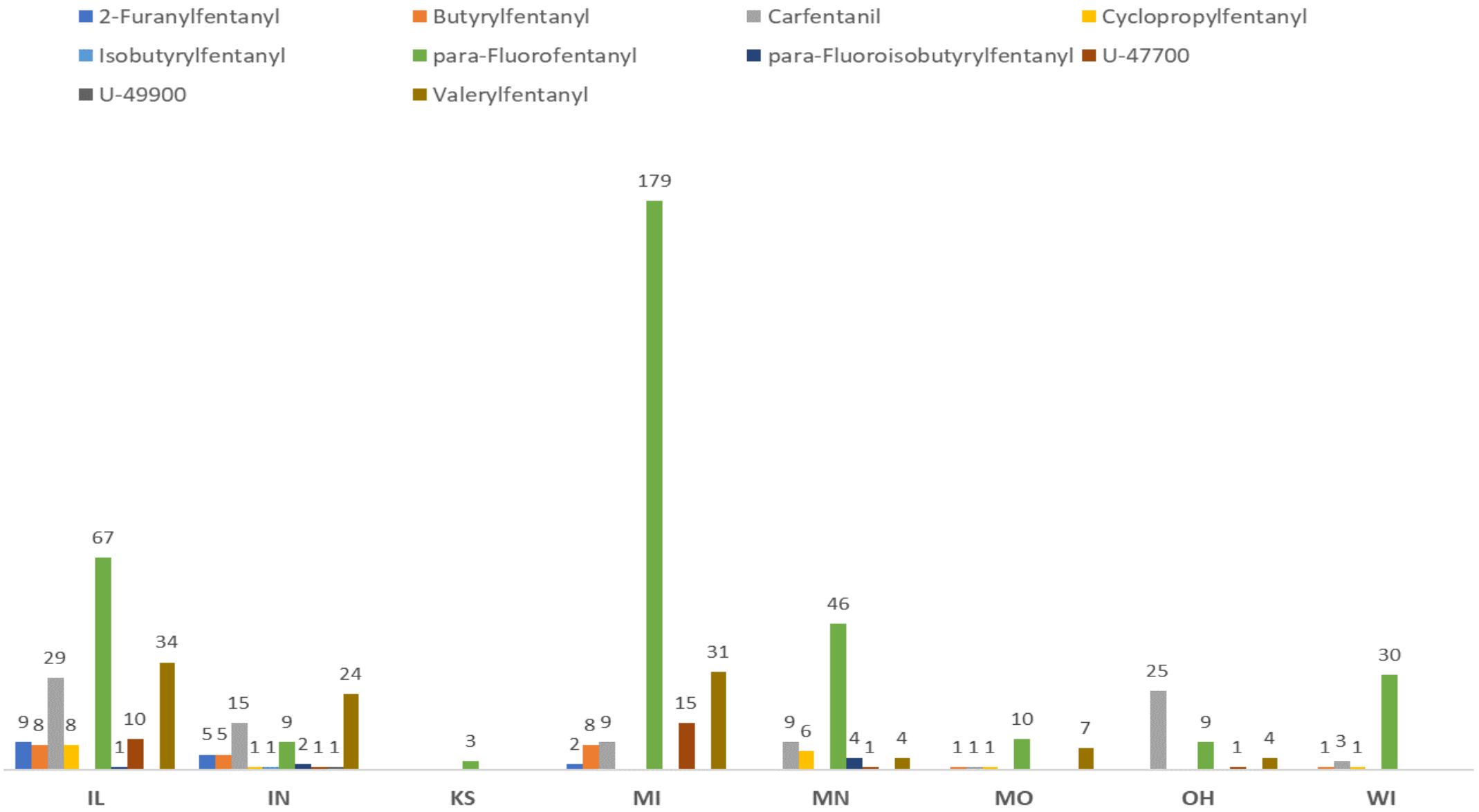
In 95% p-FF detections, fentanyl was also detected



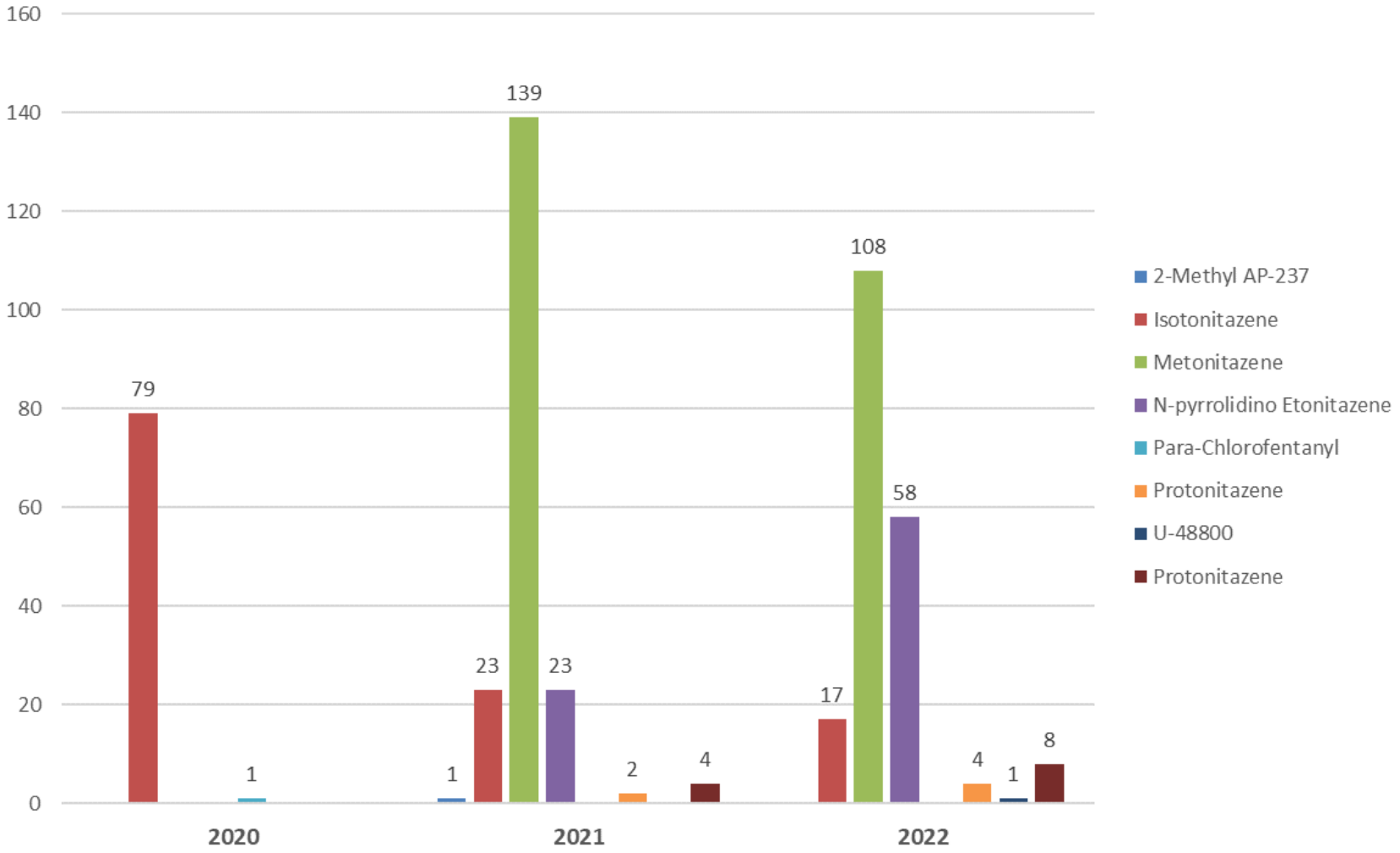


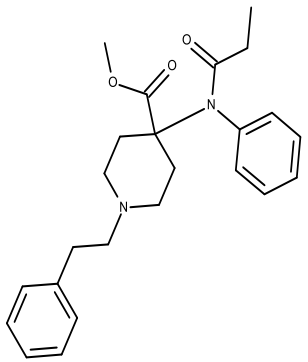
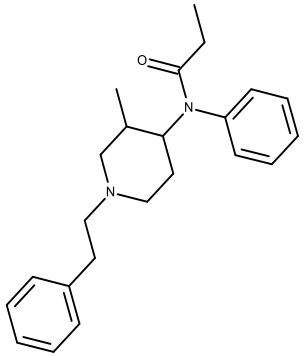
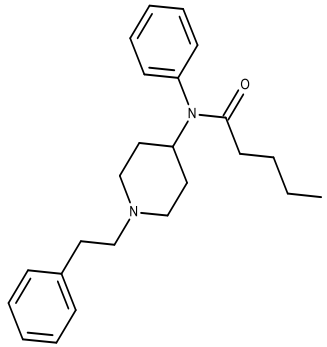
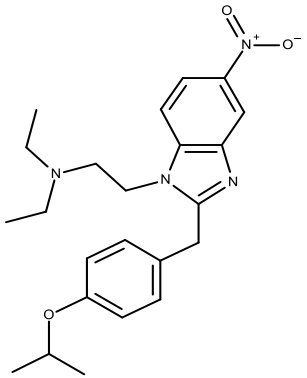
- No correlation between fentanyl-pFF concentrations

Synthetic opioids confirmations in the Midwest 2019-Aug 2021



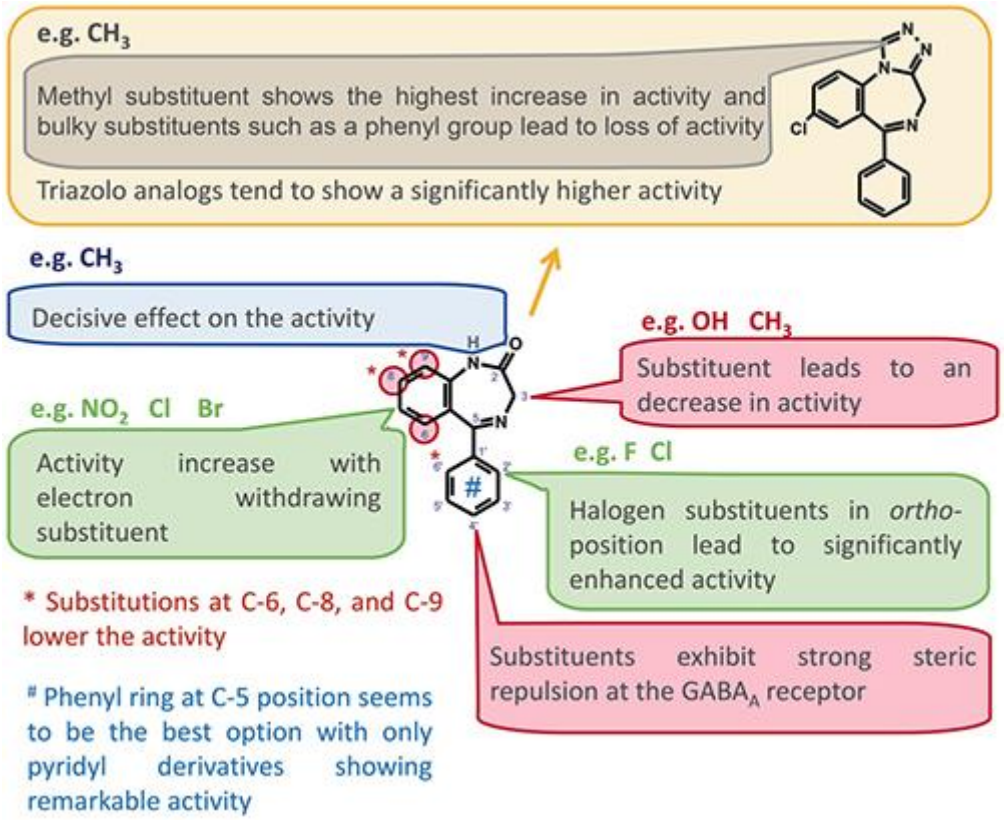
Out-of-Scope findings confirmed upon request 2020-2022, new Synthetic Opioids



					
		Carfentanil	**3-Methylfentanyl**	**Valerylfentanyl**	**Isotonitazene**
Concentration (ng/mL)	Peripheral*	Mean: 0.491 Median: 0.265 (n=46)	Mean: *cis* 0.342; *trans* 0.141 Median: cis 0.270; 0.120 (n=16)	Mean: 1.06, Median: 0.640 (n=127)	Mean: 1.41 Median: 0.875 (n=52)
	Central	Mean: 0.390, Median: 0.220 (n=134)	Mean: cis 0.805; *trans* 0.330 (n=2)	Mean: 5.11 Median: 0.51 (n=25)	Mean: 1.35 Median: 1.00 (n=7)
Analytical Methodology		Solid phase extraction, LC-MS/MS	Solid phase extraction, LC-MS/MS	Solid phase extraction, LC-MS/MS	Standard addition, LC-MS/MS
Age (Y)		Mean: 37.1, Median: 36.0 (n=414)	Mean: 37.4, Median: 40.0 (n=60)	Mean: 38.3, Median: 37.0 (n=401)	Mean: 40.7, Median: 39.0 (n=208)

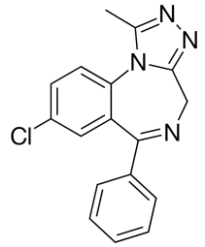
Designer benzodiazepines

Designer Benzodiazepines

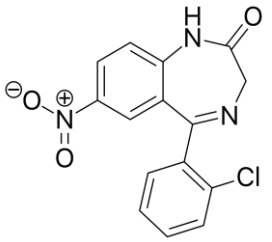


General structure-function association for different benzodiazepine modifications. Moosmann et al., Handb Exp Pharmacol 2018.

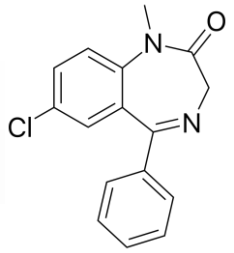
Prescription benzodiazepines are used for numerous indications such as anxiety, insomnia, muscle relaxion and epilepsy



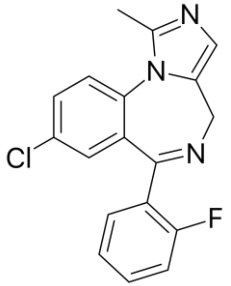
Alprazolam



Clonazepam

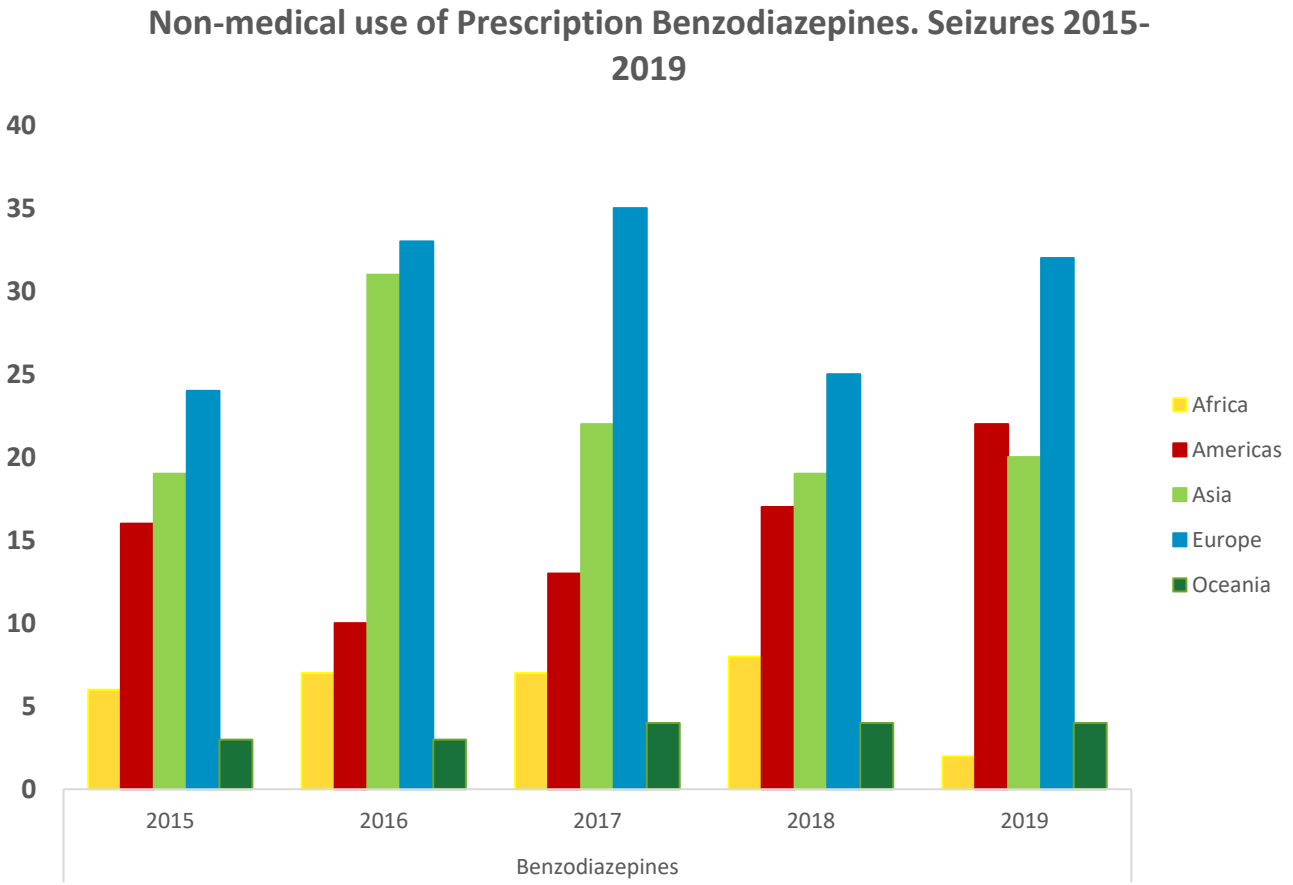


Midazolam

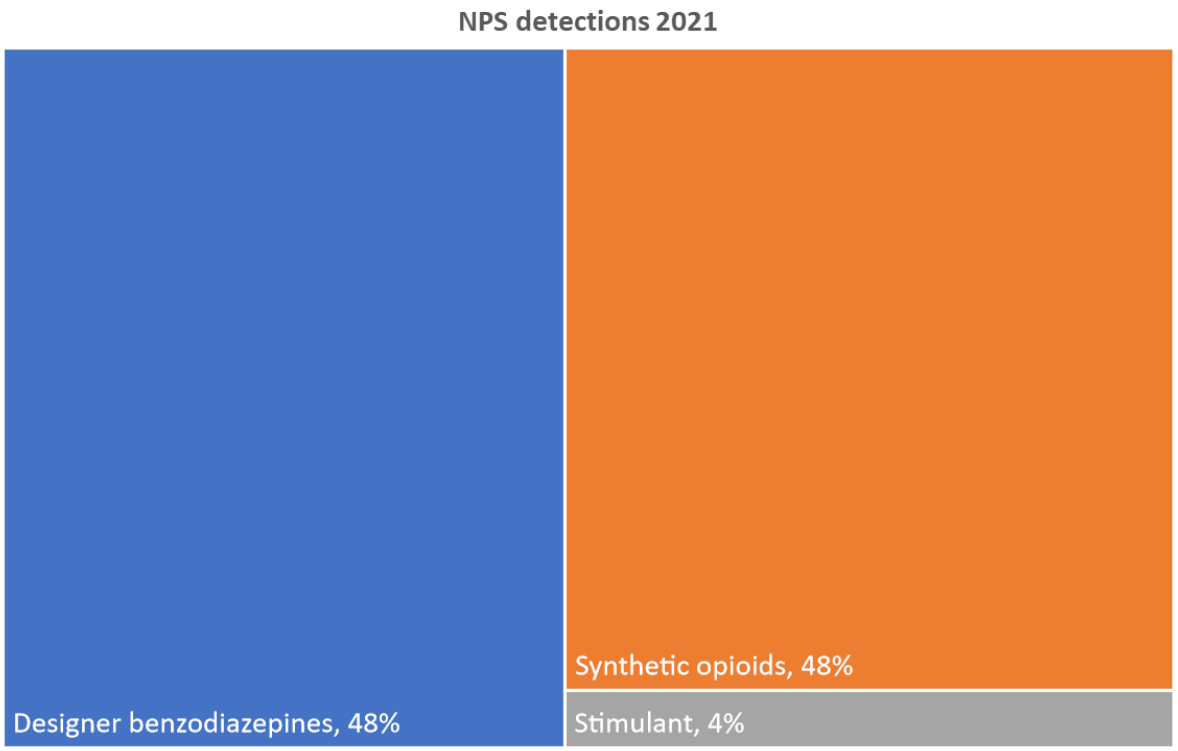


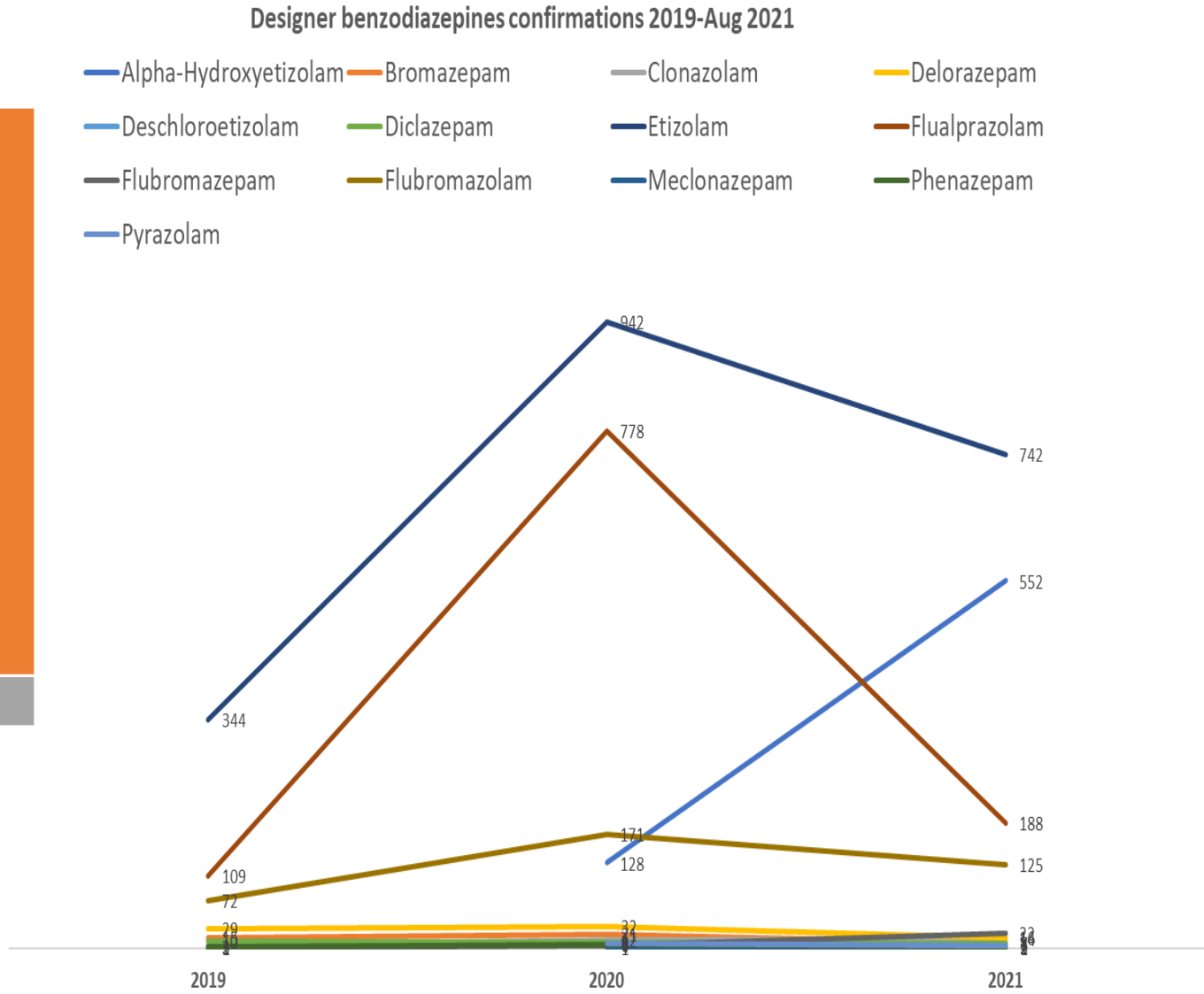
Diazepam

Designer Benzodiazepines

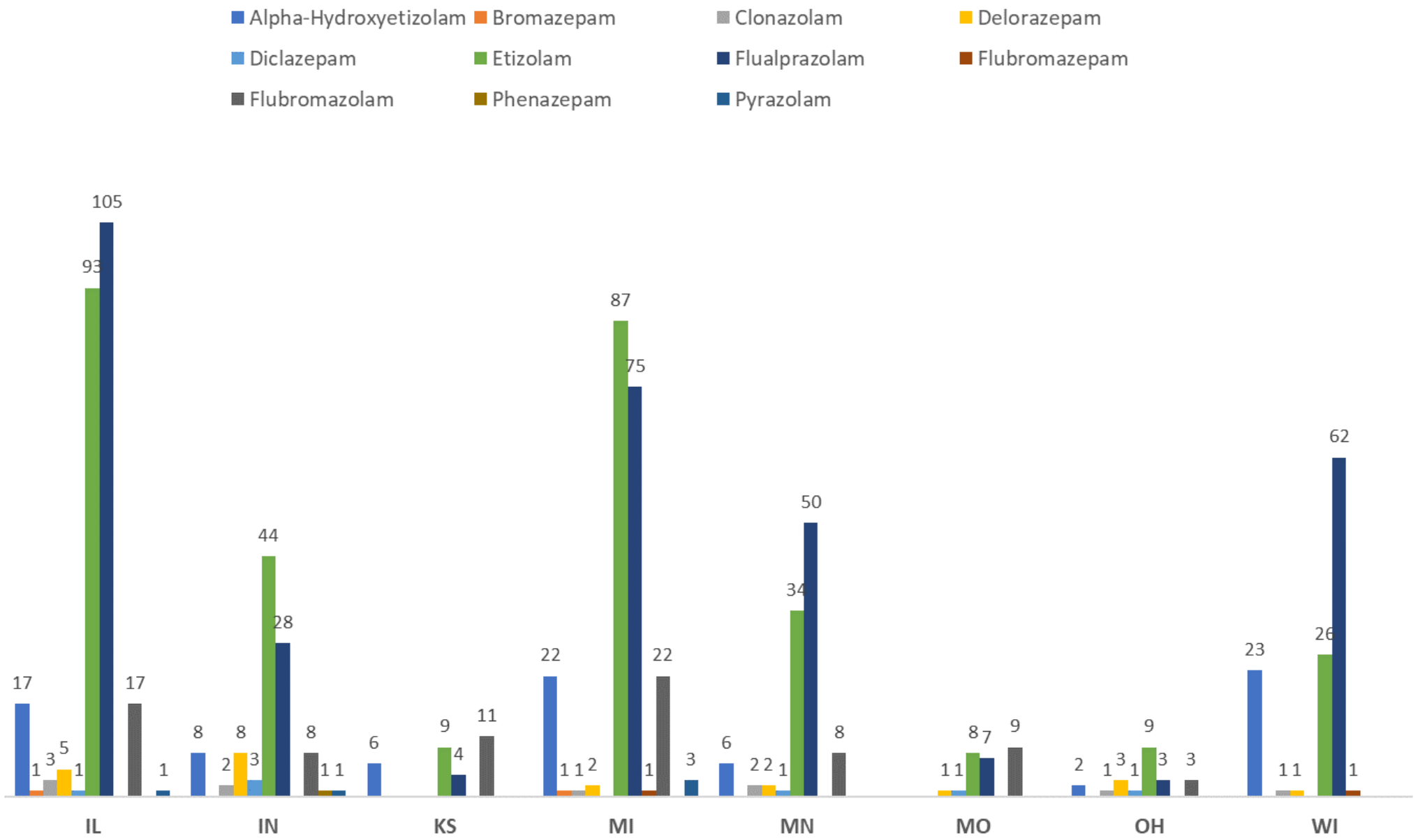


Data source: https://www.unodc.org/unodc/en/data-and-analysis/wdr2021_annex.html


NPS detections 2021
Designer benzodiazepines, 48%
Synthetic opioids, 48%
Stimulant, 4%


Designer benzodiazepines confirmations 2019-Aug 2021
Alpha-Hydroxyetizolam
Bromazepam
Clonazolam
Delorazepam
Deschloroetizolam
Diclazepam
Etizolam
Flualprazolam
Flubromazepam
Flubromazolam
Meclonazepam
Phenazepam
Pyrazolam
942
778
742
552
344
188
171
128
125
109
72
2019
2020
2021

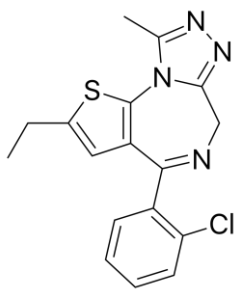
Designer benzodiazepines confirmations in the Midwest 2019-Aug 2021



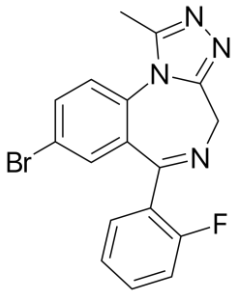
Designer Benzodiazepines

Timeline of benzodiazepines formally notified to the EU Early Warning System for the first time, 2007–2020

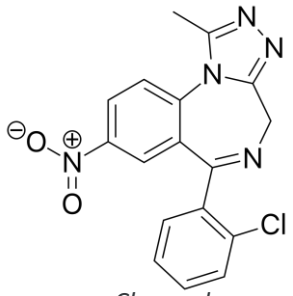
2007	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Phenazepam	Etizolam	Pyrazolam	Diclazepam Flubromazepam	Meclonazepam Deschloroetizolam Flubromazolam Alprazolam 'precursor'	Nifoxipam Clonazolam Adinazolam Metizolam Nitrazolam	Cloniprazepam 3-hydroxyphenazepam Fonazepam 4-chlorodiazepam Flunitrazolam Bromazolam	Norfludiazepam Ro 07-4065 Thionordazepam	Methylclonazepam Flucotizolam Tofisopam Flualprazolam Clobromazolam	Bentazepam Cinazepam	



Etizolam
(2011)



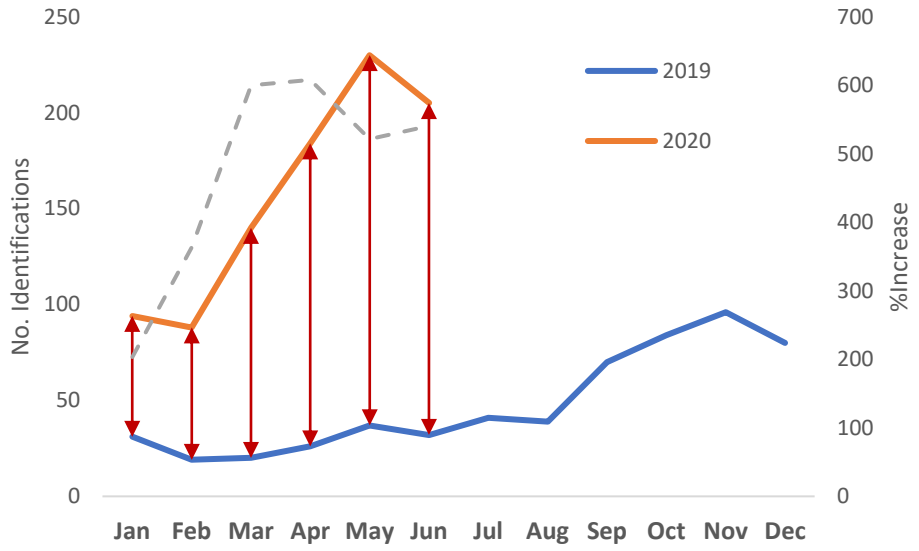
Flubromazolam
(2014)

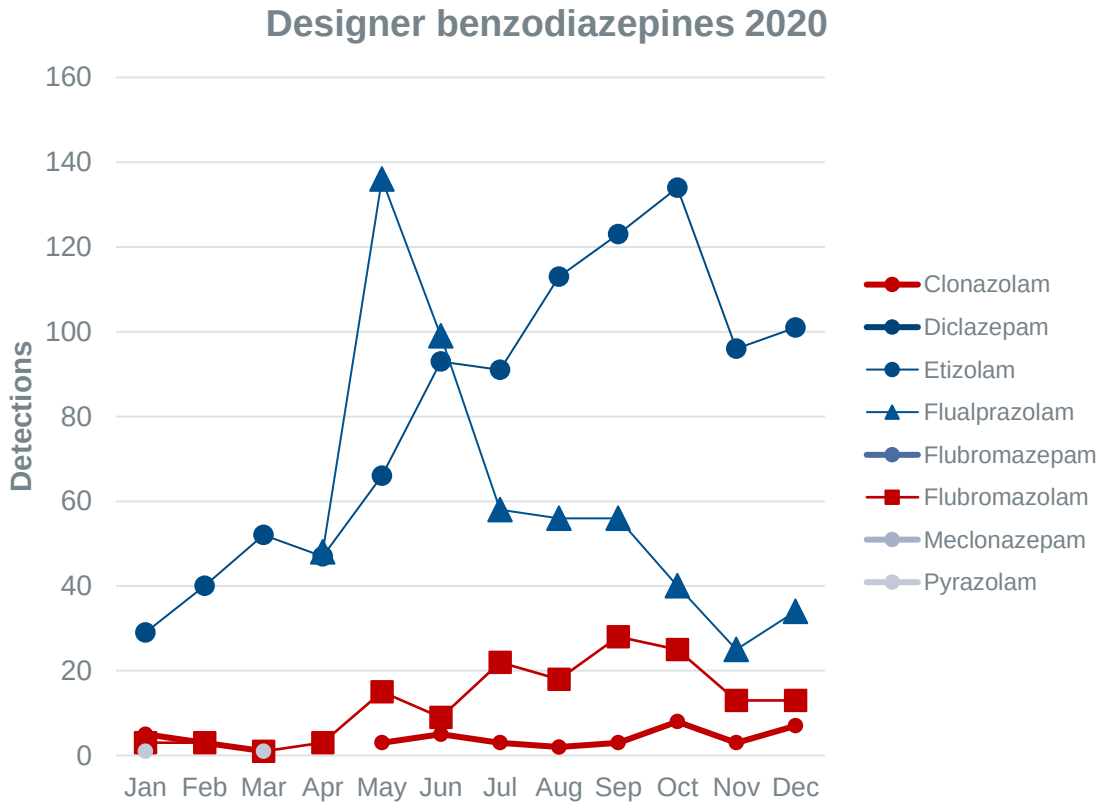


Clonazolam
(2015)

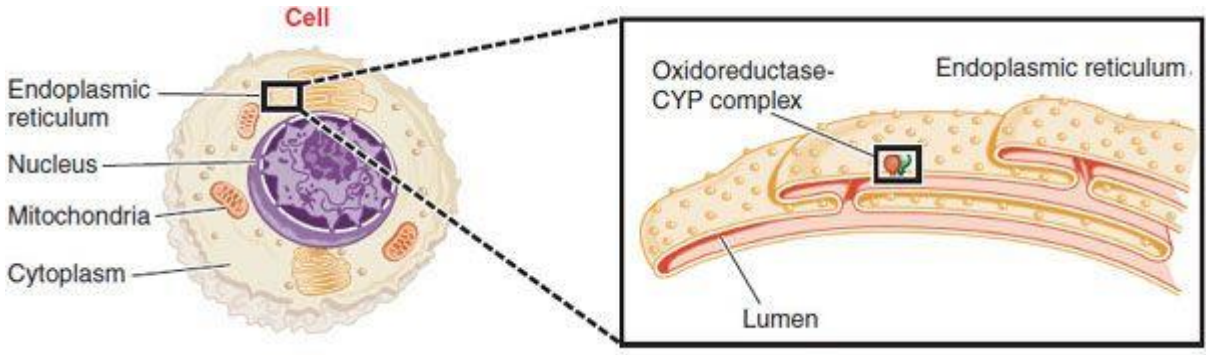
Source: EMCDDA, https://www.emcdda.europa.eu/system/files/publications/13759/TD0221596ENN_002.pdf

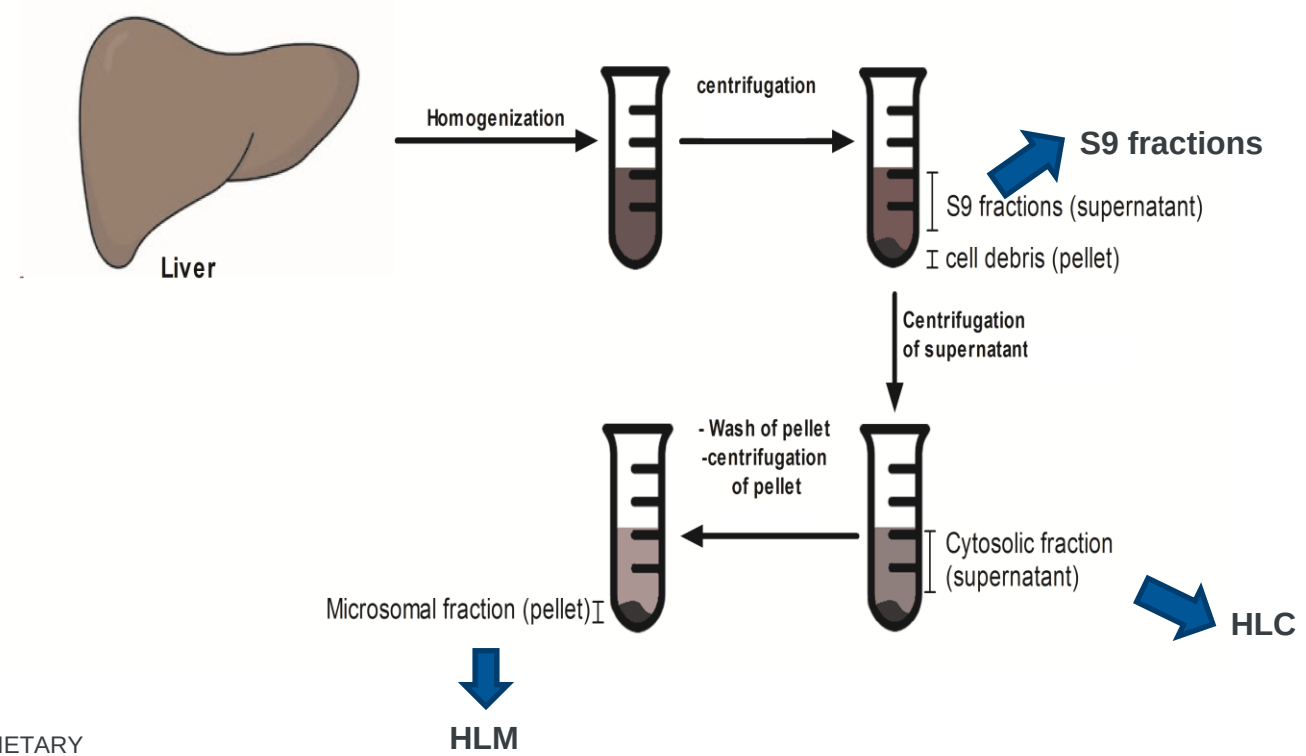
Designer Benzodiazepines





In vitro Metabolism Studies





Models

- S9 fractions
- Cytosol
- Microsomes

Method

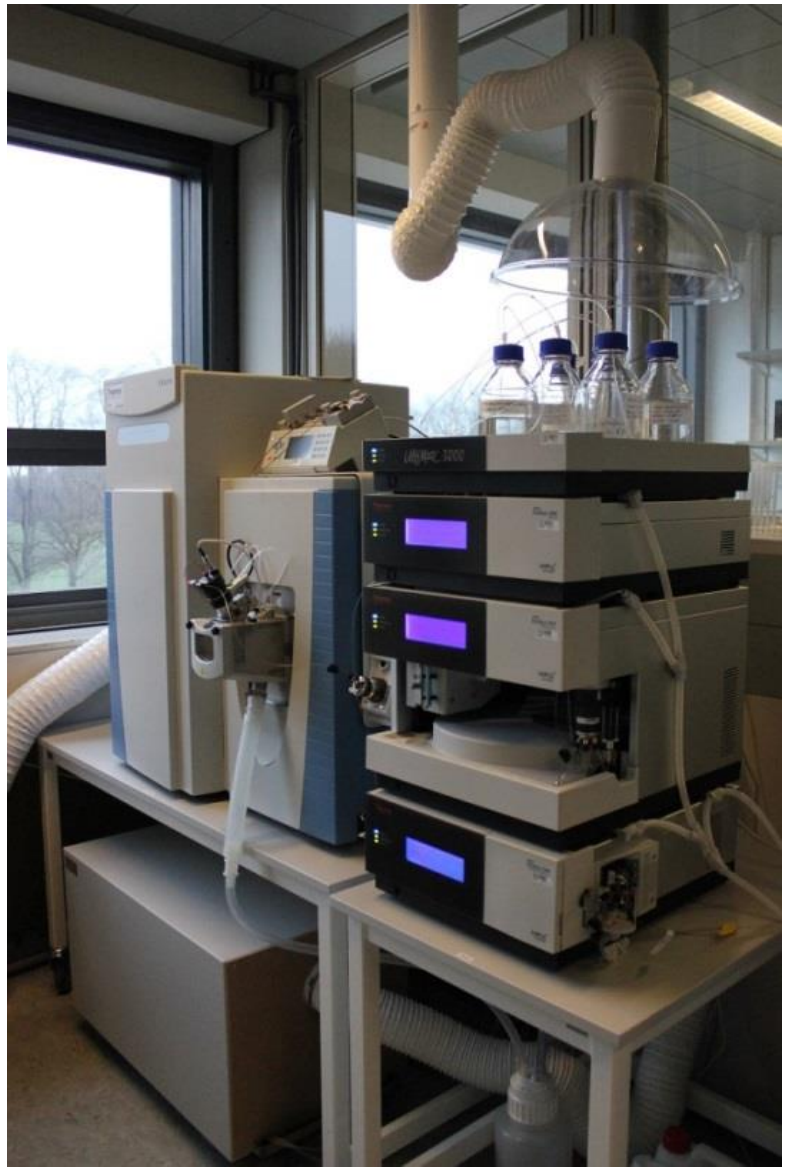
30 μL

Incubator

- ✓ [Substrate] = 5 μM
- ✓ pHLM 1 mg protein/mL or
- ✓ rCYP-enzyme 50 pmol/mL
- ✓ (+/-)NADPH, (+/-)UDPGA
- ✓ Total 180 min of incubation at 37 °C
- ✓ (+/-)Enzyme inhibor

Quench plate

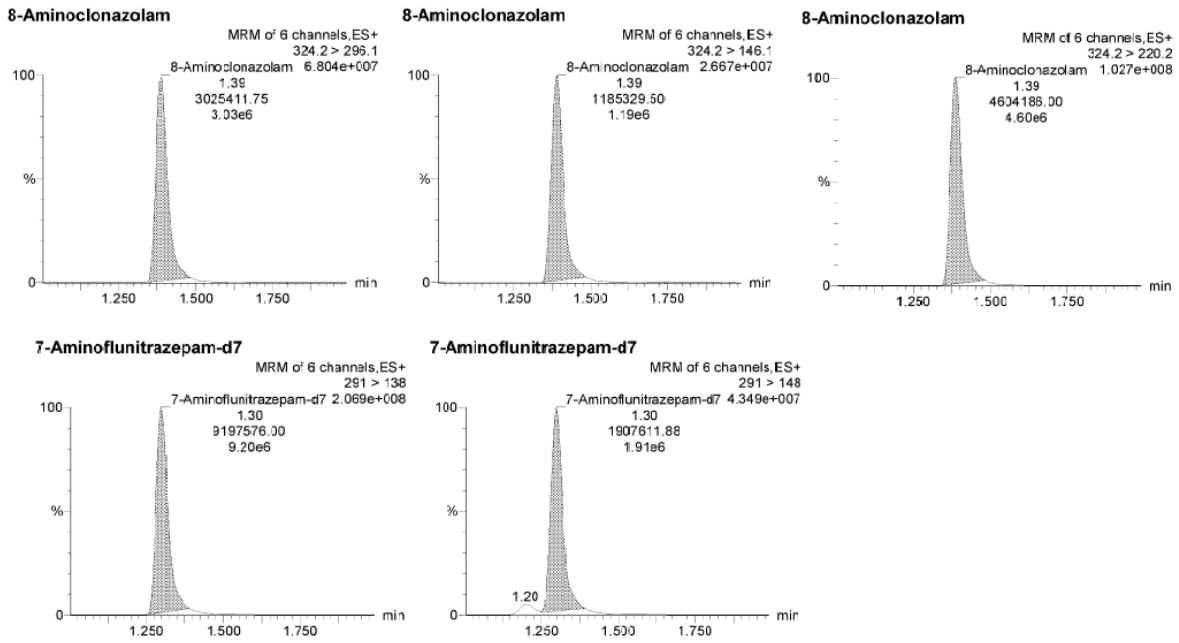
- ✓ 60 μL 100 ng/mL ISTD in ACN



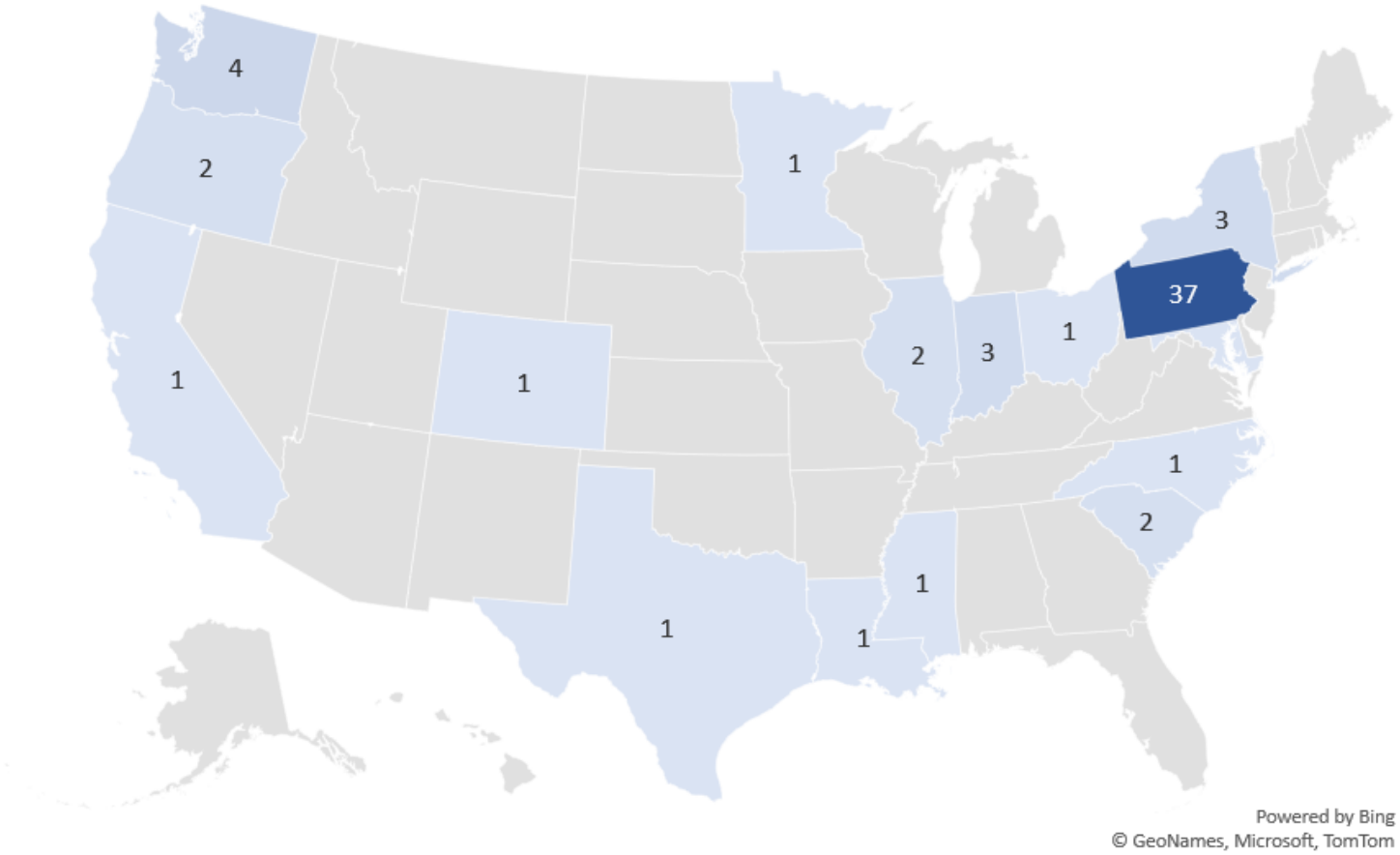
LC-HRMS, Thermo Scientific Q Exactive™

Confirmation of 8-Aminoclonazolam

- Calibration range: 2-200 ng/mL
- ISTD: 7-Aminoflunitrazepam-d7
- Extraction via a protein precipitation with acetonitrile set up following with SPE



Clonazolam


4
2
1
1
1
1
3
37
2
3
1
1
2
1
1
1
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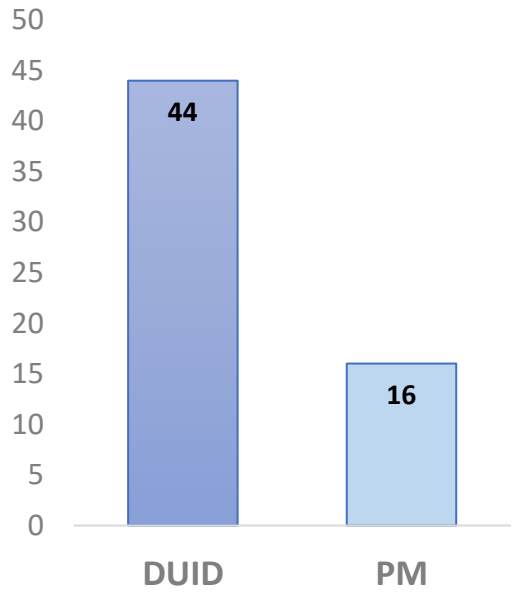
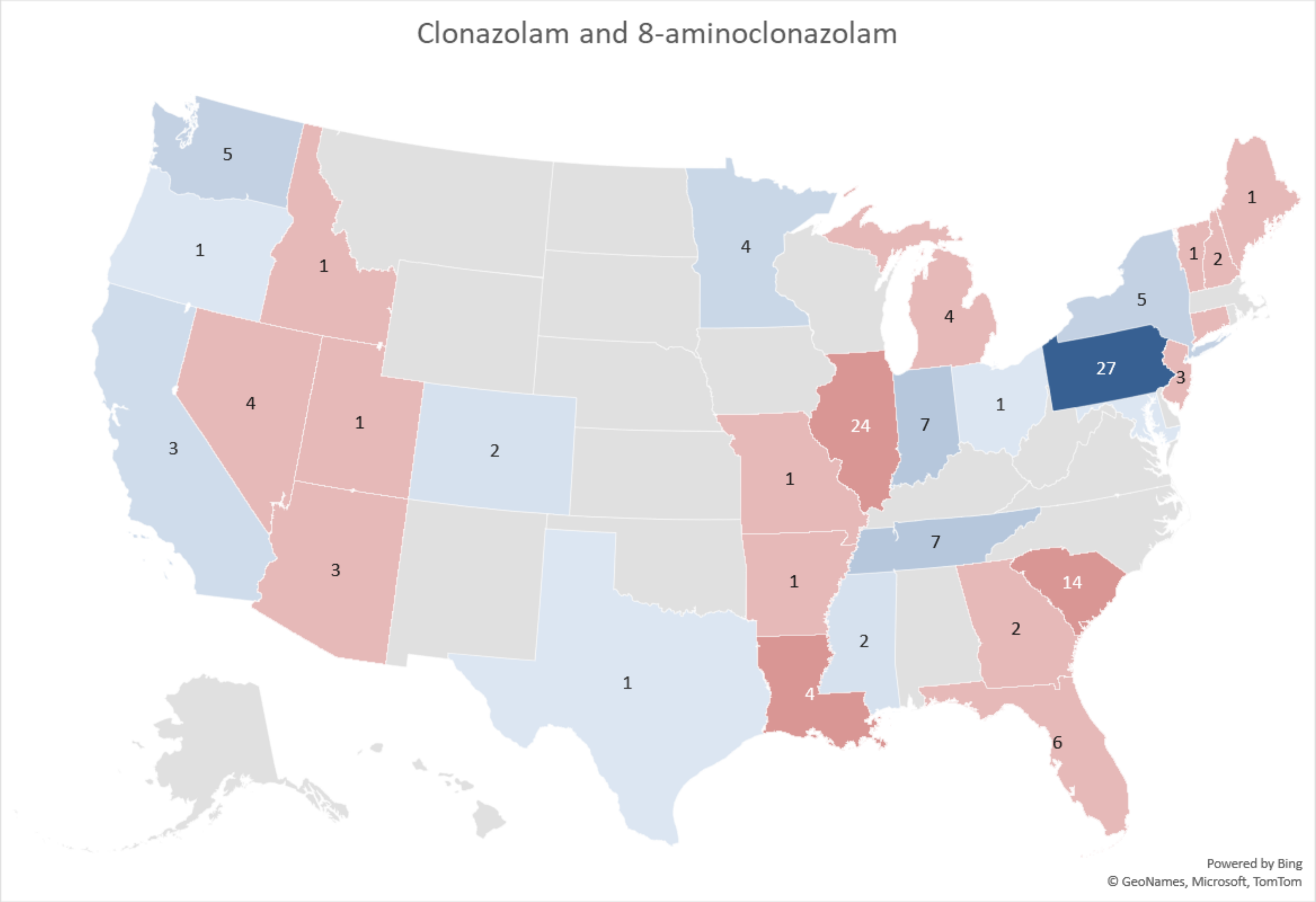

50
45
40
35
30
25
20
15
10
5
0
44
16
DUID
PM

Clonazolam



Clonazolam and 8-aminoclonazolam
5
1
1
4
1
3
1
2
4
3
1
4
24
7
1
27
3
5
1
2
1
7
1
14
2
2
4
6
1
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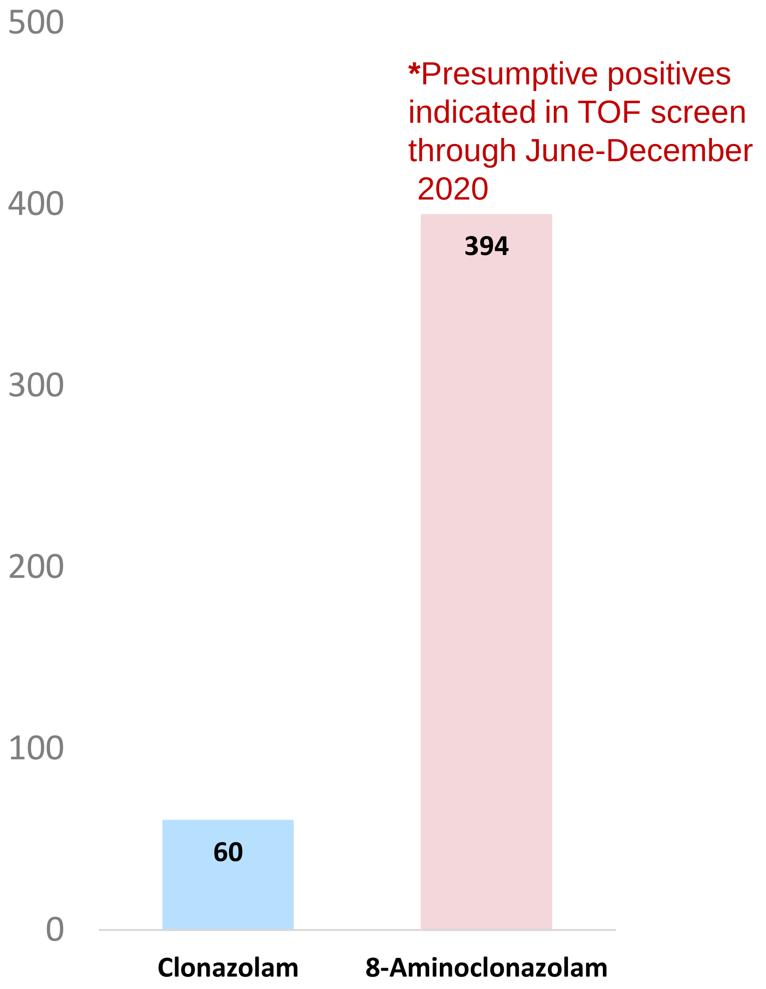
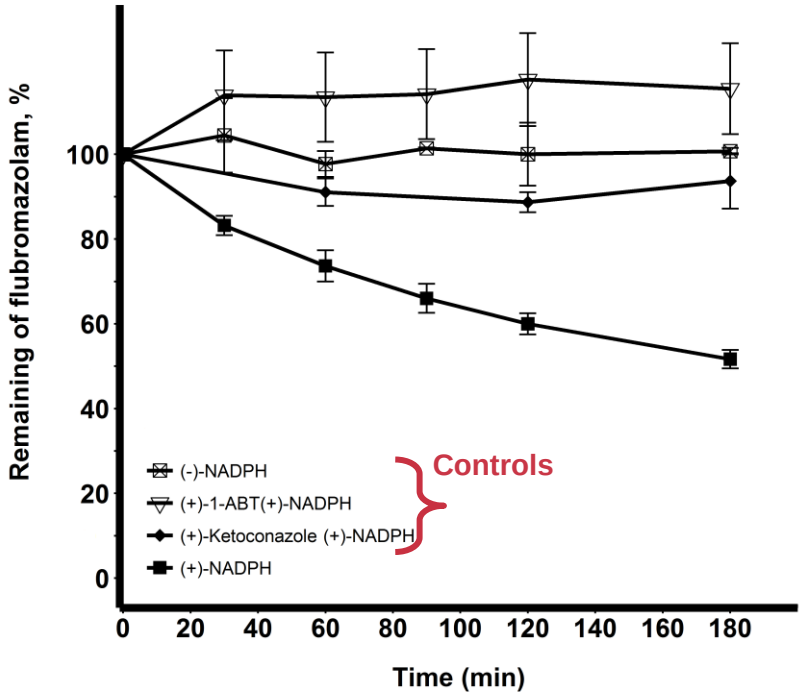
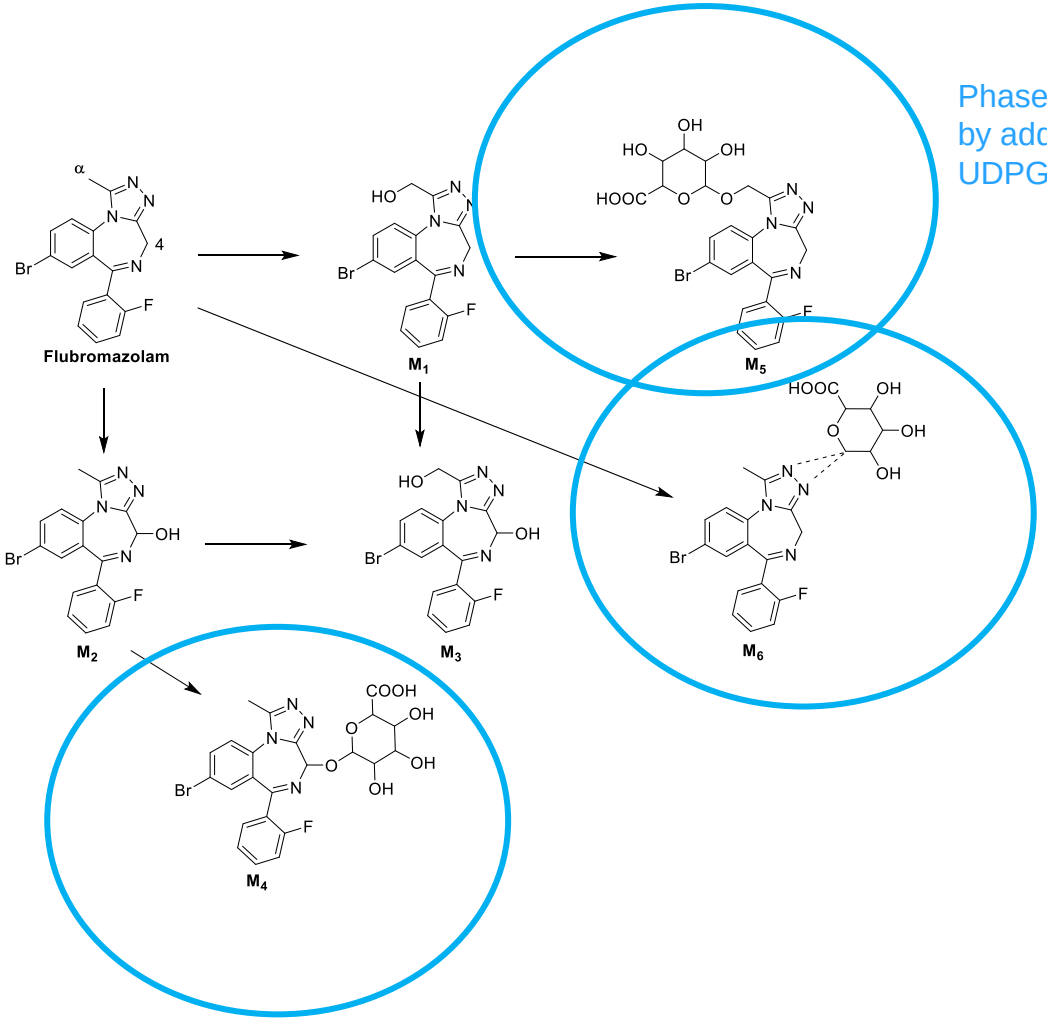


500
400
300
200
100
0
*Presumptive positives indicated in TOF screen through June-December 2020
394
60
Clonazolam
8-Aminoclonazolam

	Peripheral blood concentration (ng/mL)			
	Median	Mean	Range	N
Clonazolam	8.1	11	5.0–86	43
8-Aminoclonazolam	64	117	9.9–570	16

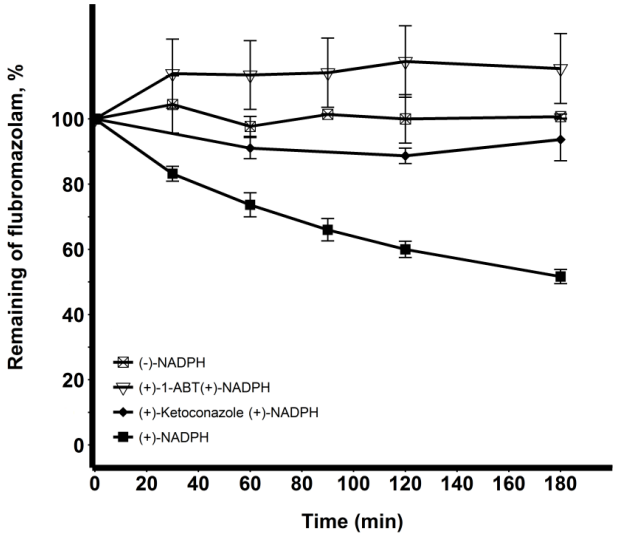
Other example of in vitro metabolism studies: Flubromazolam



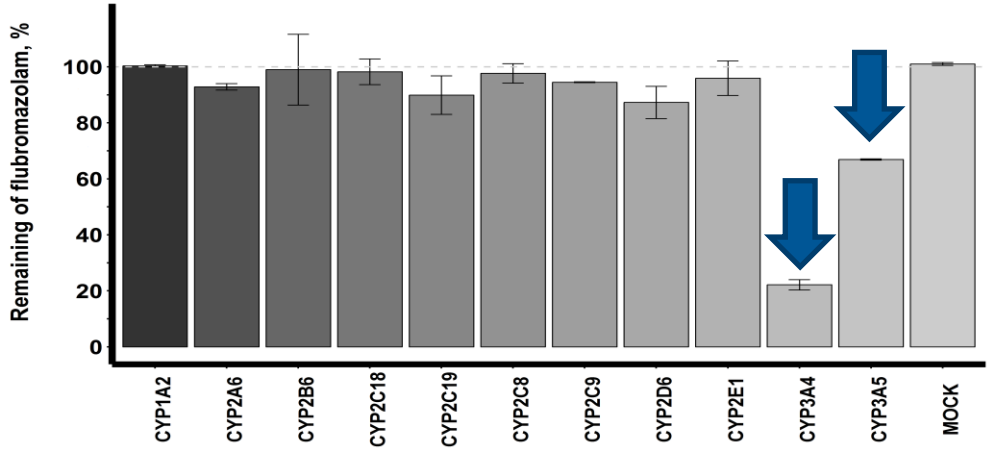


Phase II metabolism by adding cofactor UDPGA

Other example of in vitro metabolism studies: Flubromazolam



Reaction phenotyping



NADPH-dependent metabolism

CYP3A-mediating metabolism predominates in HLM

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