

# Designer Benzodiazepine Positivity and Co-Positivity in Urine Specimens

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## Introduction

- Designer Benzodiazepines (DBZD) are synthetic substances that produce similar pharmacological effects as prescription benzodiazepines, though are not approved for use in the United States of America (USA).
- DEA reported a 157% increase in identifications of DBZD in 2020 compared to 2019.<sup>1</sup>
- Flualprazolam and etizolam accounted for approximately 81% of all DBZD identifications followed by clonazolam and flubromazolam at approximately 11 and 3%, respectively.<sup>1</sup>
- Flualprazolam has been identified in counterfeit alprazolam tablets.<sup>2</sup>
- Use of DBZD will go undetected when testing for traditional prescription benzodiazepines by mass spectrometry therefore, including testing for DBZD in at risk populations may provide a more complete picture of a patient's substance use history and allow for more informed care.

## Objective

- To evaluate DBZD positive urine specimens to determine prevalence of specific DBZD, co-positivity with other drugs/drug classes, and patient demographics including age and sex.

## Methods

- DBZD were analyzed as part of a larger Novel Psychoactive Substances (NPS) panel
- From December 2020 to April 2021, 28,900 samples requesting NPS testing were submitted from 36 states in the continental USA

### Sample preparation

- Hydrolysis with liquid-liquid extraction followed by evaporation and reconstitution

### Chromatographic separation

- LC-MS/MS: reverse phase, Restek Raptor Biphenyl (100x3mm, 2.7µm) column

### Instrumentation

- MS/MS: SCIEX API 4000™

### Data Interpretation Software

- MS/MS: SCIEX MultiQuant™ 2.1.2

- Samples positive for DBZD were evaluated for co-positivity with other drugs/drug classes.

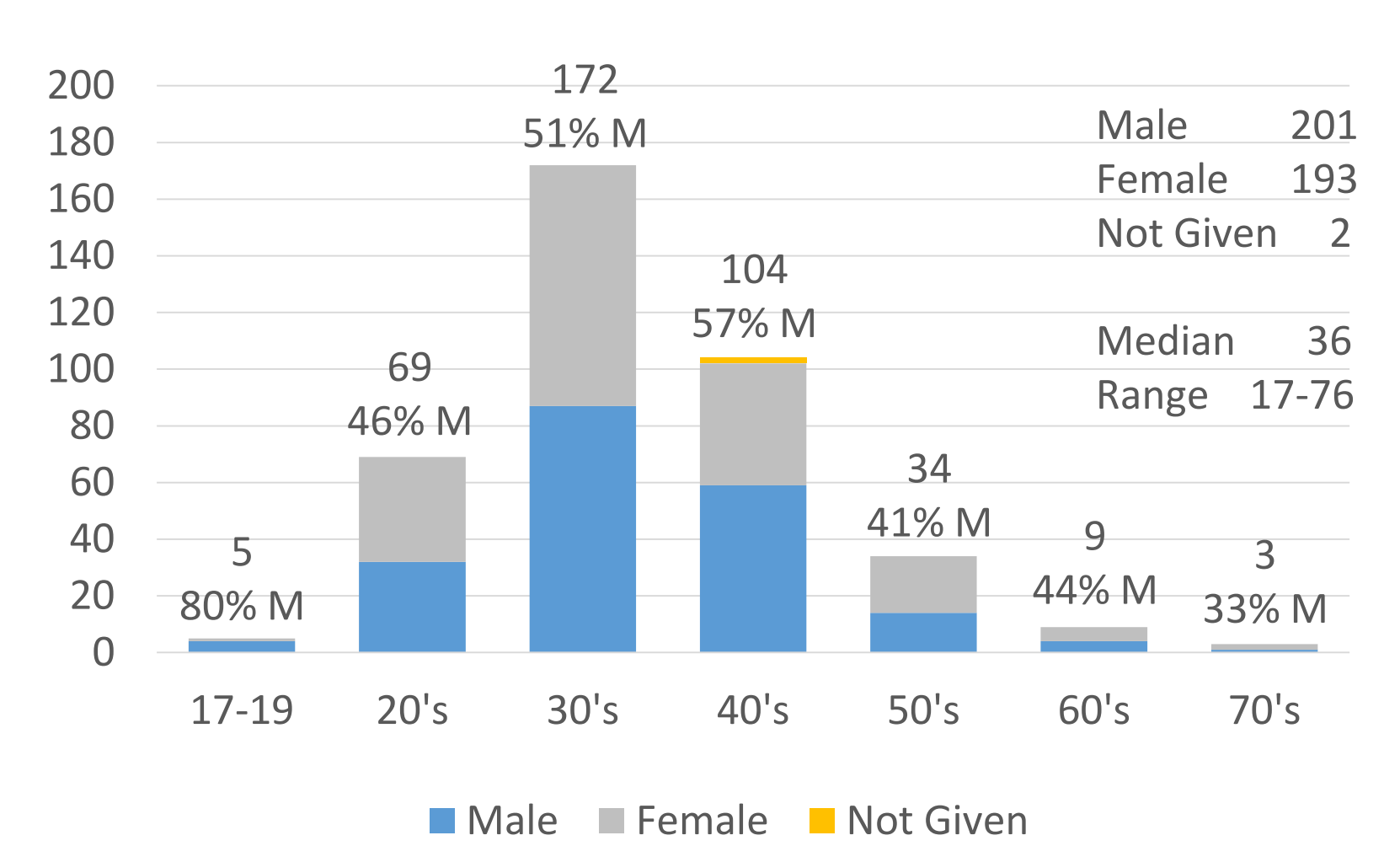
<sup>1</sup>Drug Enforcement Administration (DEA) 2020 Annual Emerging Threat Report  
<sup>2</sup>DEA 2020 National Drug Threat Assessment

## Results

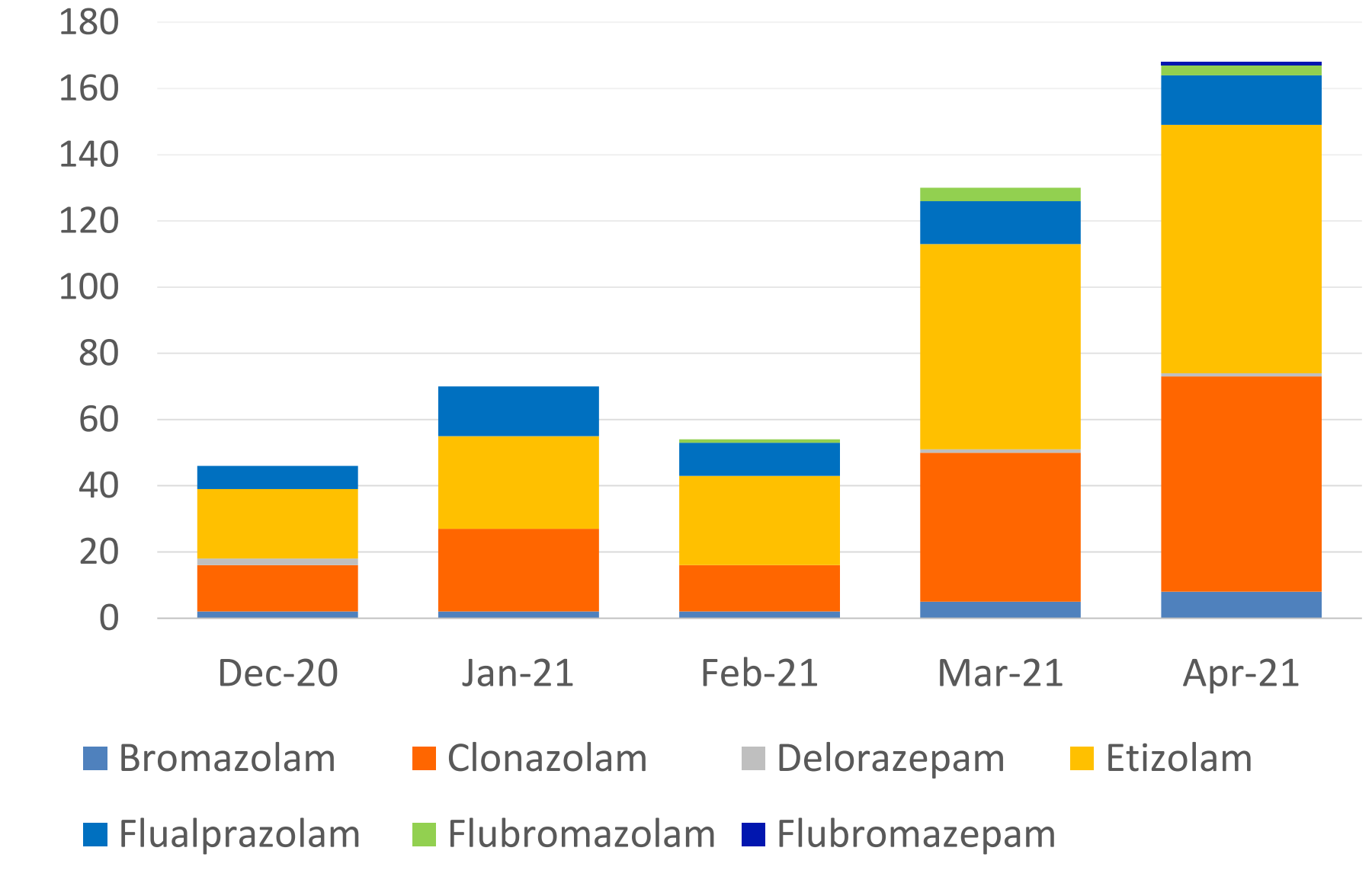
% DBZD Positivity By Month

Month	# of DBZD Positives	# Specimens ordered	% Positivity
DEC 20	46	3,651	1.3
JAN 21	70	4,098	1.7
FEB 21	54	4,005	1.3
MAR 21	130	7,109	1.8
APR 21	168	10,037	1.7

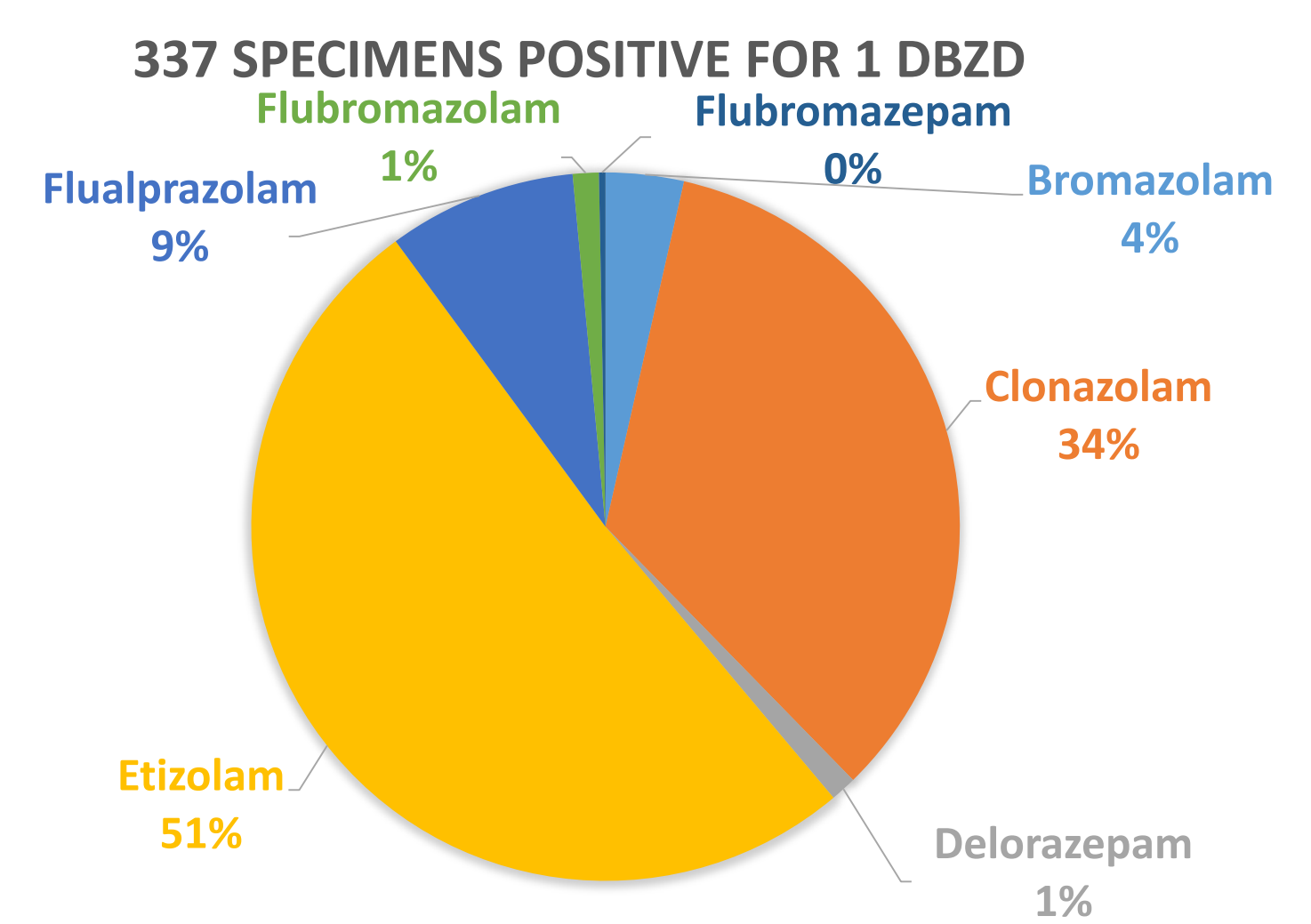
Patient Age and Sex



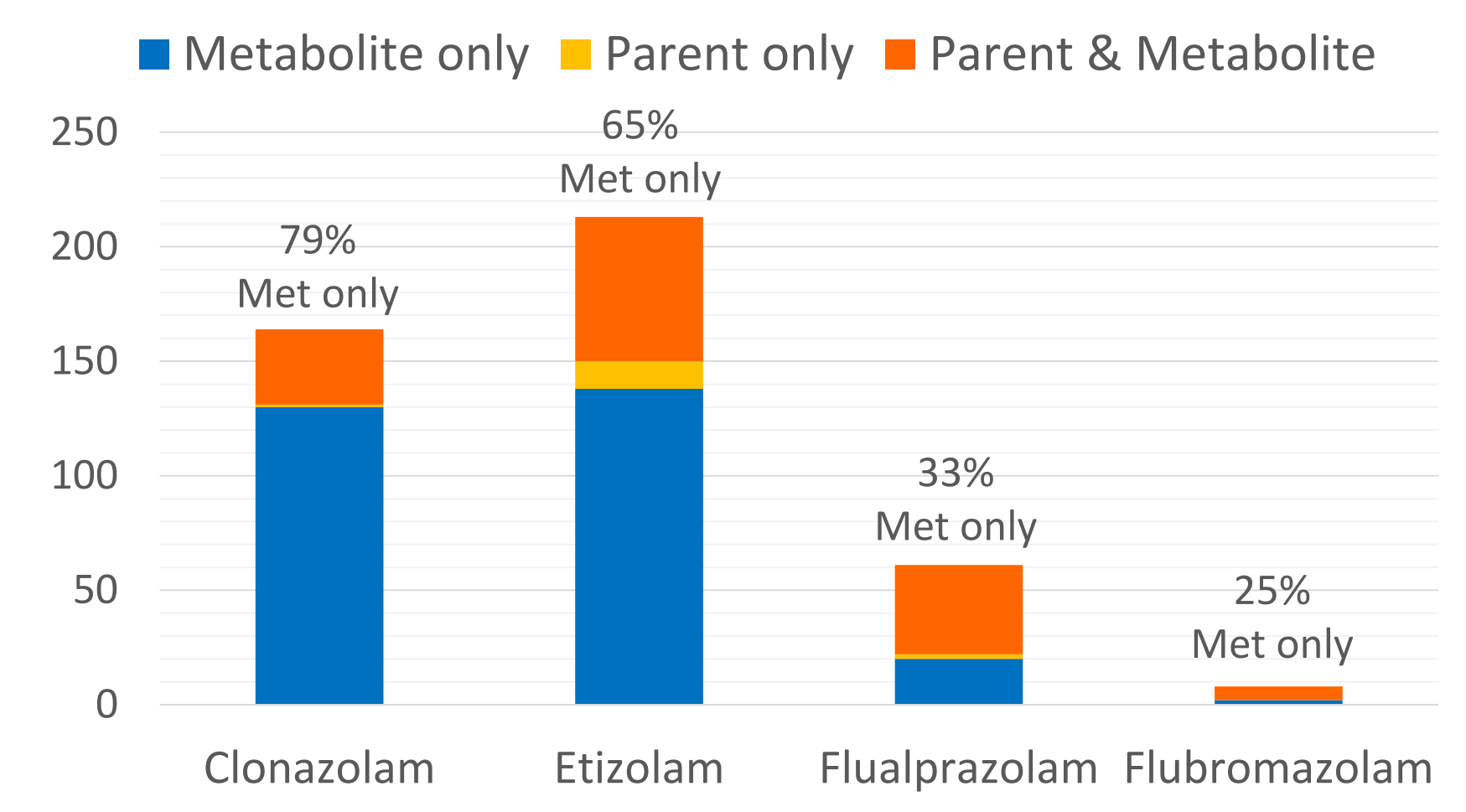
DBZD Positive By Month



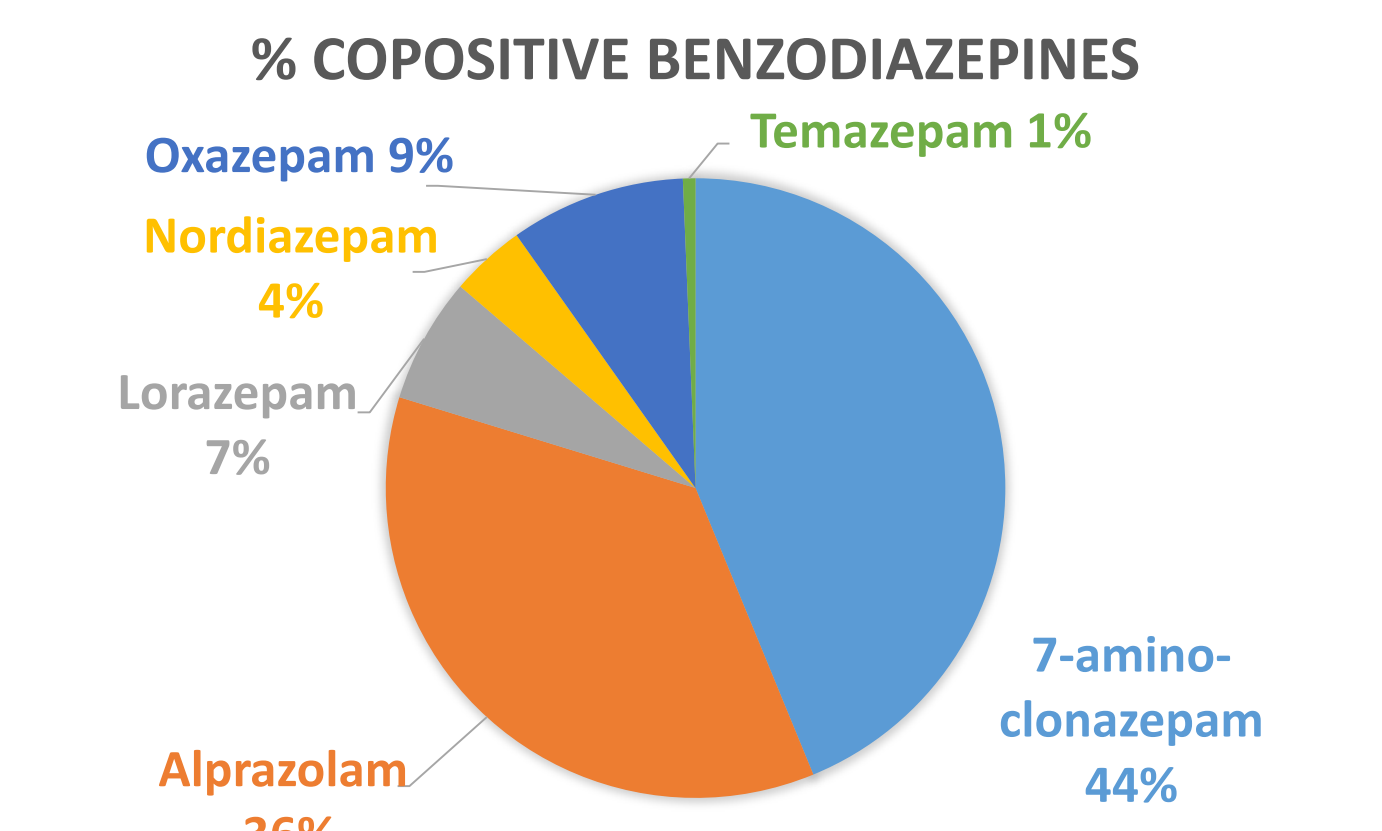
Prevalence of DBZDs



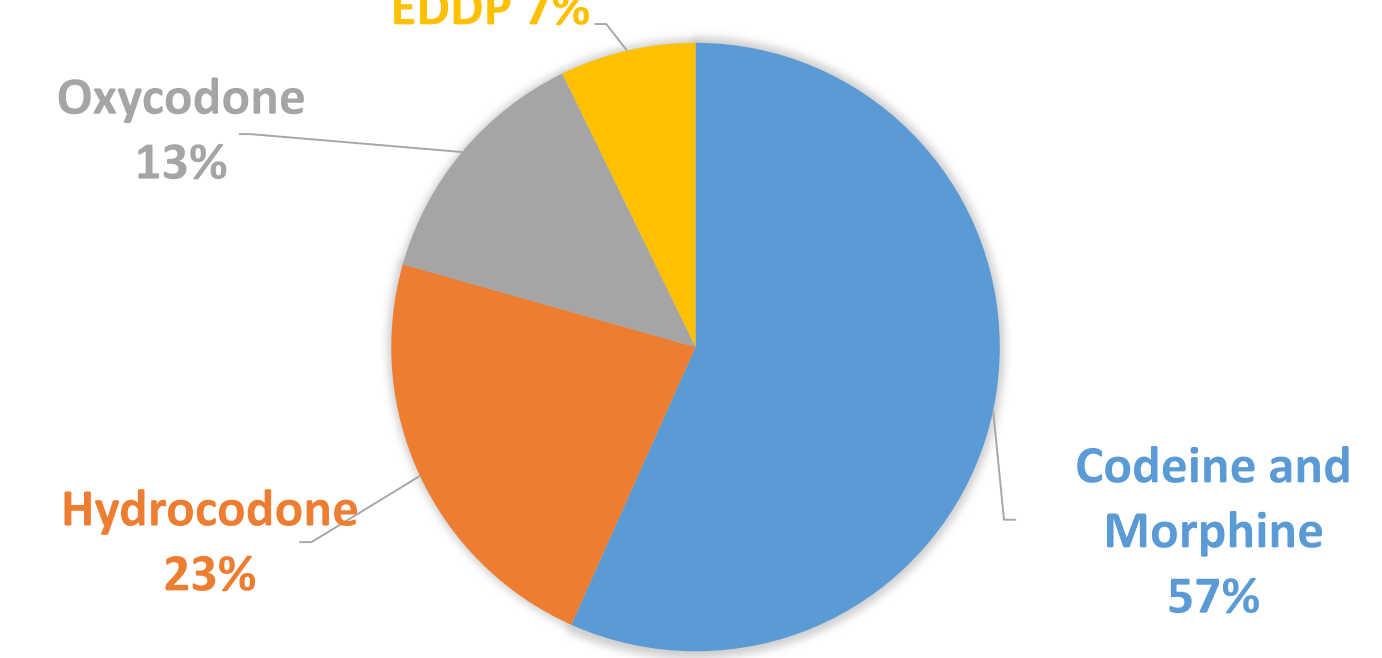
DBZD % Metabolite



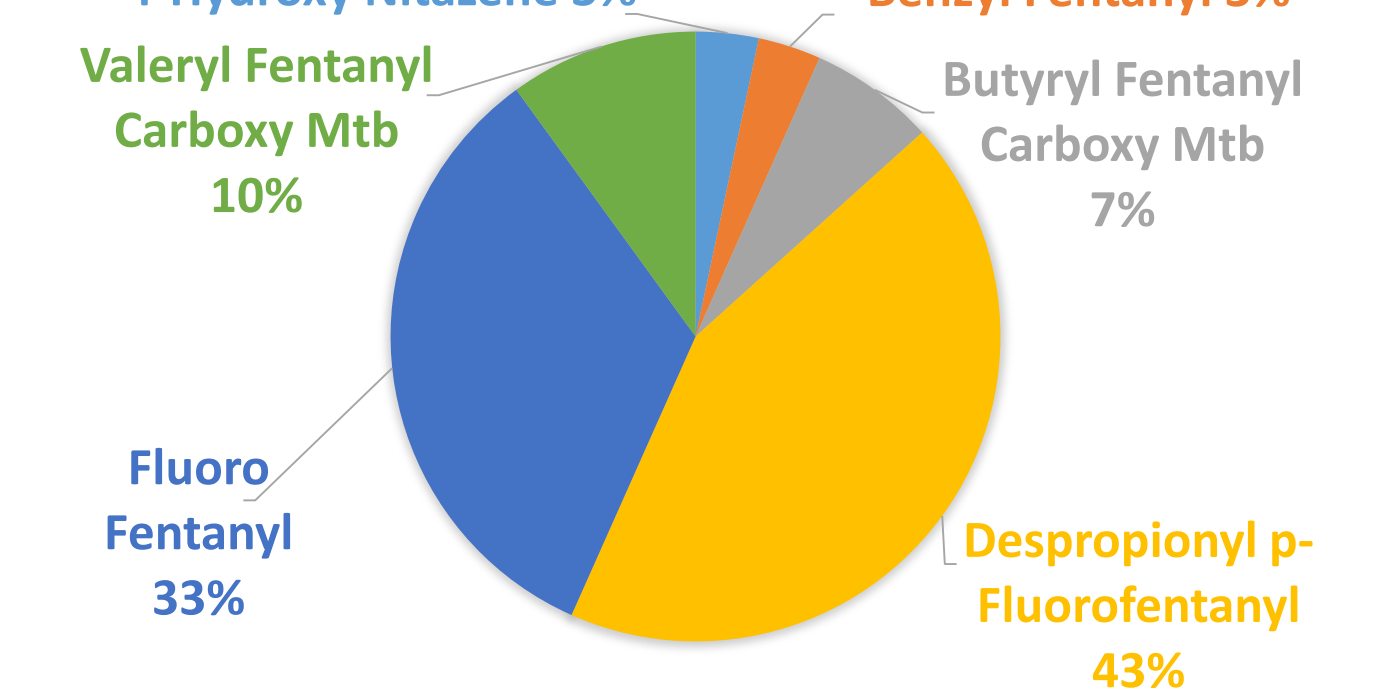
Co-Positive Drug Class By Analyte



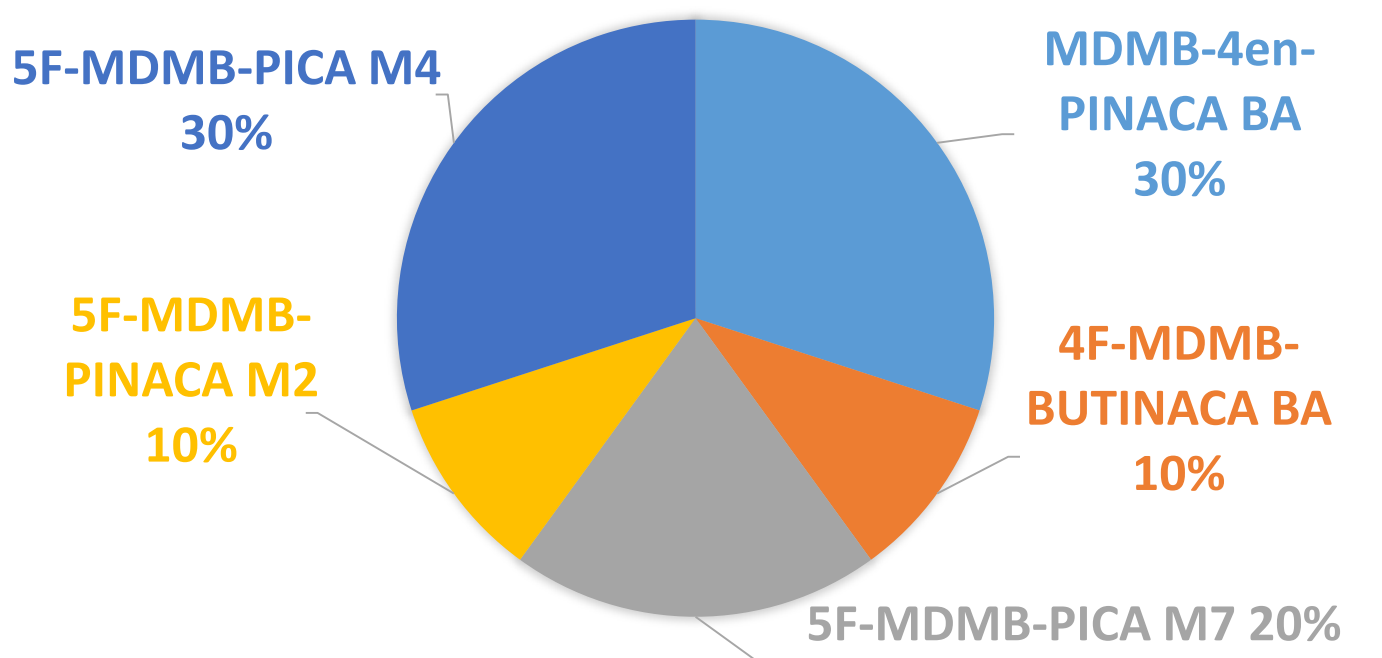
% COPOSITIVE OPIATE/OPIOIDS



% COPOSITIVE DESIGNER OPIOIDS



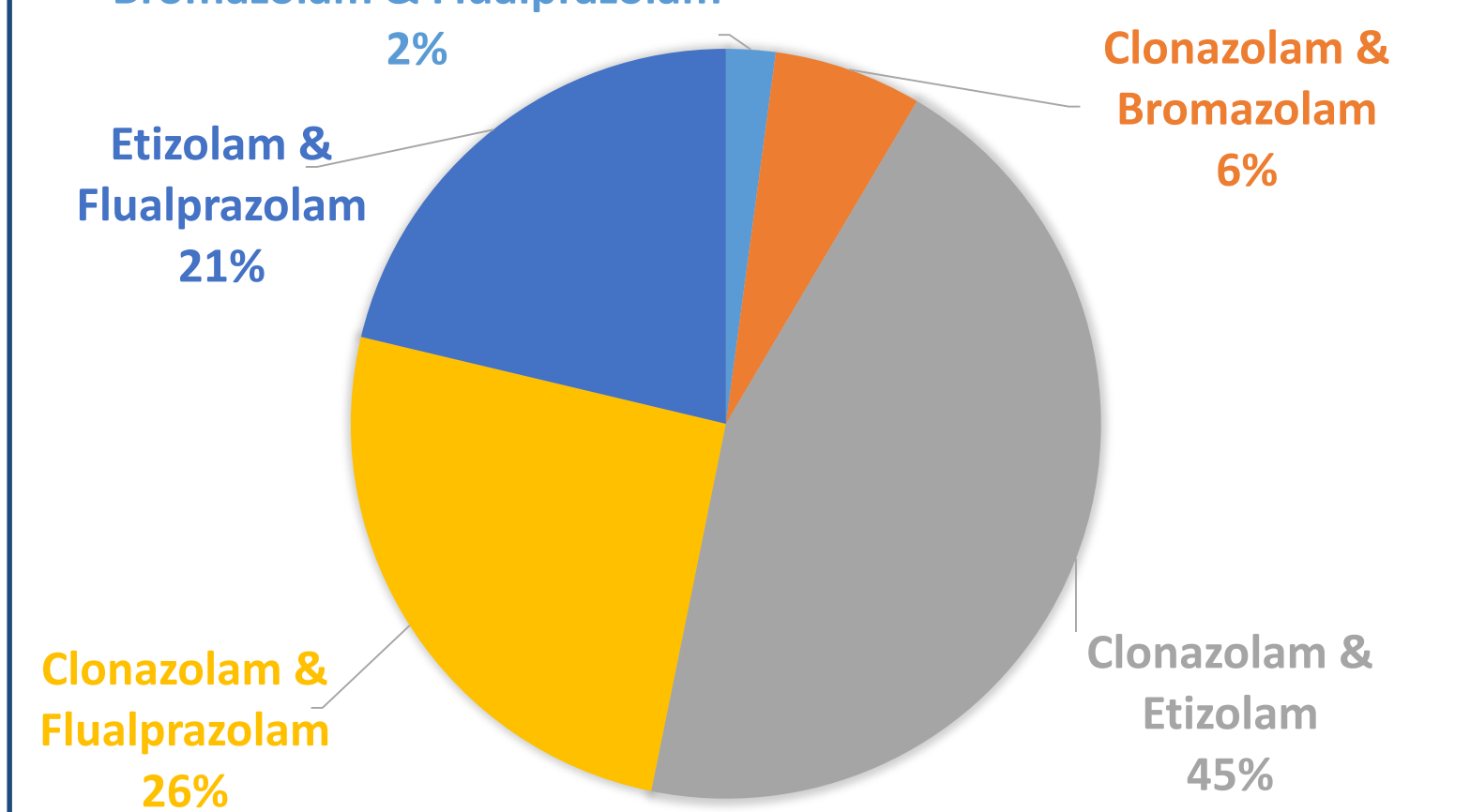
% COPOSITIVE SYNTHETIC CANNABINOIDS



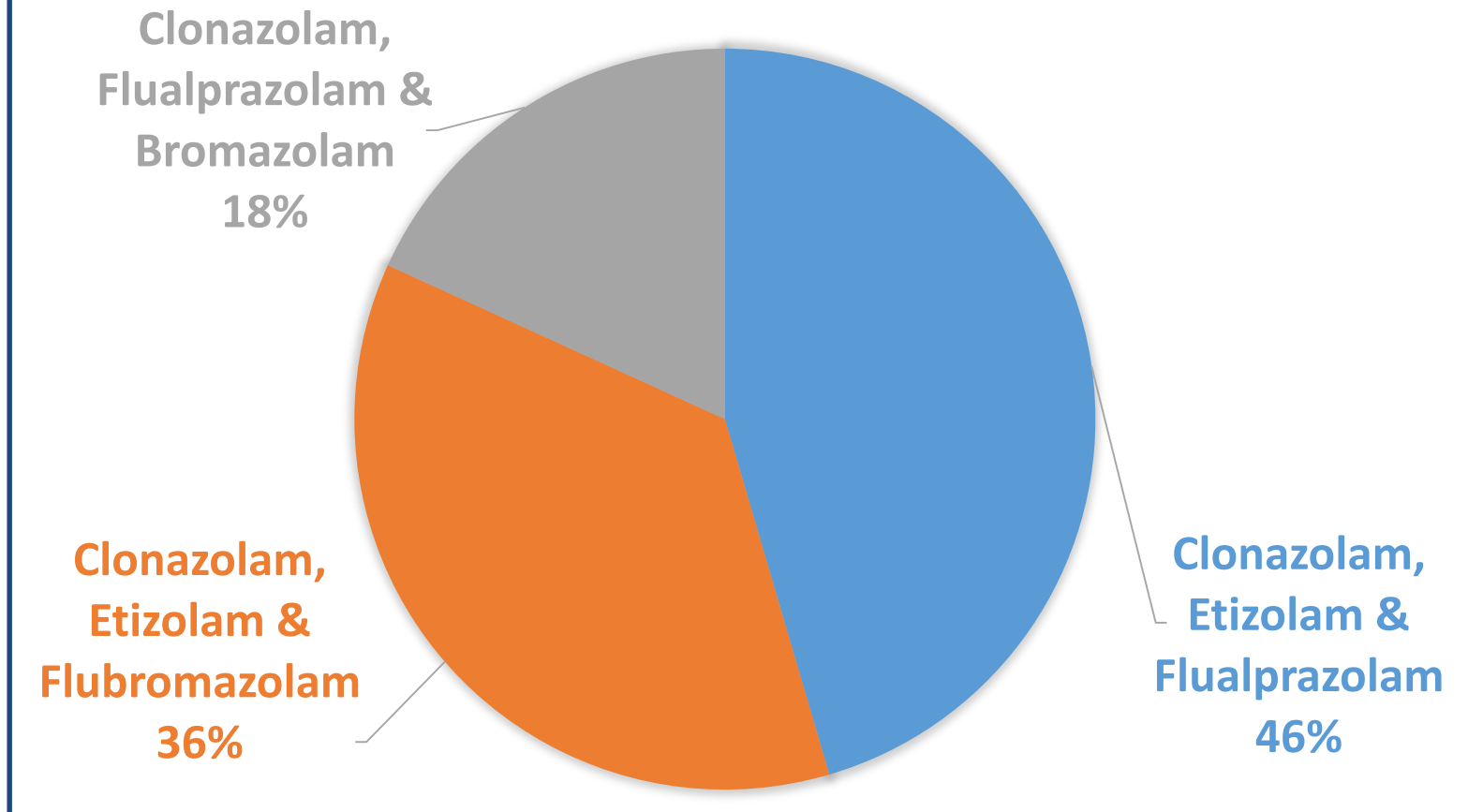
% Co-Positivity by Drug/Drug Class

Drug/Drug Class	N	%
Buprenorphine	243	61
THC	155	39
Benzodiazepines	153	38
Opiate Antagonists	153	38
Cotinine	127	32
Fentanyl	112	28
Opiates/Opioids/Methadone	97	24
Methamphetamine	82	21
Gabapentin/Pregabalin	78	20
Alcohol Metabolites	67	17
Antidepressants	57	14
Amphetamine	53	13
Cocaine	49	12
Antipsychotics	40	10
Heroin	22	6
Designer Opioids	30	8
Muscle relaxants	11	3
Synthetic Cannabinoids	10	3
Anxiolytics	9	2
Methylphenidate	8	2
Anticonvulsant	6	2
Sedative/Hypnotic	6	2
Kratom	5	1
Clonidine	5	1
Synthetic Stimulants	4	1

47 SPECIMENS POSITIVE FOR 2 DBZD



11 SPECIMENS POSITIVE FOR 3 DBZD



1 SPECIMEN POSITIVE FOR 4 DBZD

clonazolam, etizolam, flualprazolam and bromazolam

## Methods Continued

- Qualitative analysis detection limits below

DBZD	LOD (ng/mL)
Bromazolam	1
Clonazolam	1
8-aminoclonazolam	1
Diclazepam	1
Delorazepam	2
Etizolam	1
alpha-hydroxyetizolam	1
Flualprazolam	1
alpha-hydroxyflualprazolam	2
Flubromazepam	2
Flubromazolam	1
alpha-hydroxyflubromazolam	2
Nitrazepam	1
Phenazepam	2
3-hydroxyphenazepam	5

## Discussion

- Overall DBZD analyte positivity in the five month study period was 1.6% (range 1.3-1.8%)
- Overall 51% male vs 49% female with some differences by age observed.
- Median patient age 36 (range 17 to 76). The majority of positives were for patient's in their 30's (43%) and 40's (26%). Thus designer drugs are not only used by younger persons.
- 396 (1.4%) of 28,900 specimens were DBZD positive
- 337 specimens (85% of DBZD positives) were positive for a single DBZD with etizolam (51%) and clonazolam (34%) being the most prevalent.
- 59 specimens (15% of DBZD positives) were positive for more than one DBZD.
- Clonazolam and Etizolam were detected by metabolite only in 79% and 65% of positives respectively. Thus it is important to include metabolites of these analytes in analytical methods testing for these compounds in urine.
- The highest co-positivity was buprenorphine at 61%. The high co-positivity of opiate antagonists at 38% may be due to use of buprenorphine/naloxone combination products.
- This study demonstrated patients use DBZD with other benzodiazepines, opioids including fentanyl, heroin, and designer opioids, medications used to treat opioid addiction, and other CNS depressants which represent risk of overdose and death.
- Thus including testing for DBZD in at risk populations may allow for more informed care.