

Emergence of the Novel Opioid N-Desethyl Isotonitazene in the Recreational Drug Supply

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Joshua DeBord*, Sara Walton, Donna Papsun, Alyssa Reyes, Barry Logan, Alex Krotulski



DISCLOSURES

- I am a paid employee of CFSRE
- I have no conflicts of interest in the material of this presentation
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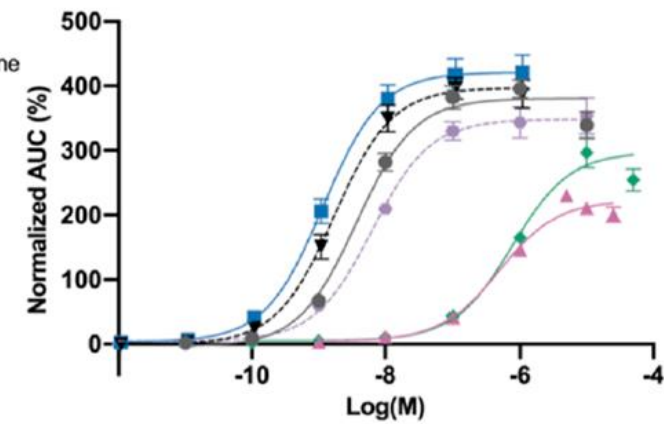
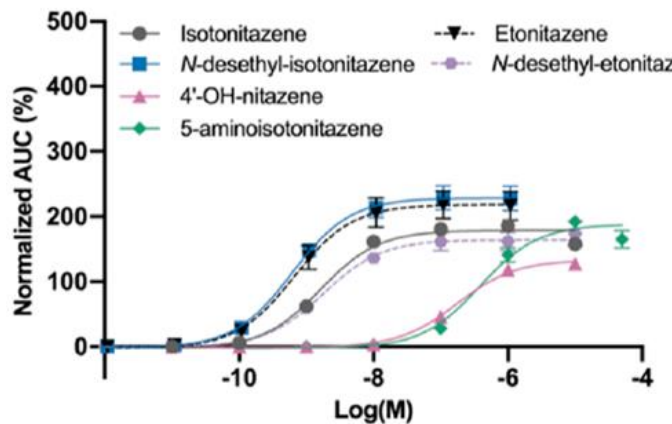
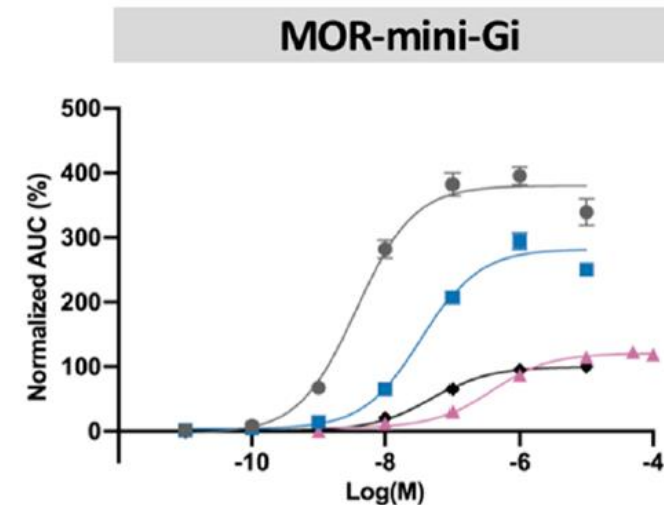
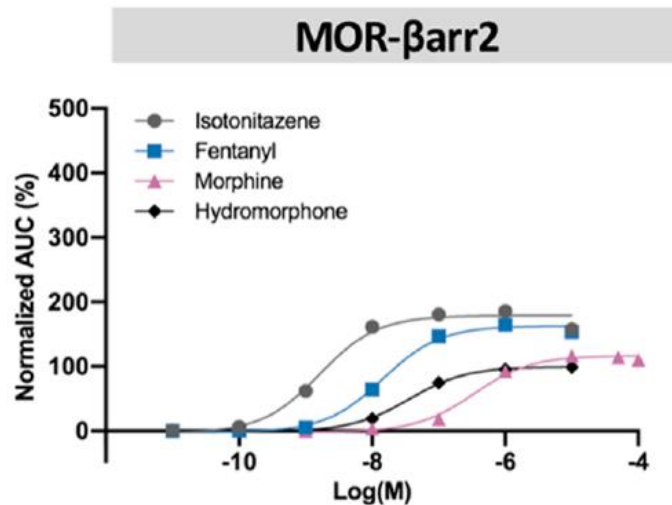


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INTRODUCTION

- Nitazene analogs, “Zenes”, synthetic opioid class of novel psychoactive substance (NPS)
- Isotonitazene: 2019
- N-Desethyl Isotonitazene (NDI): 2022
- ~50x more potent than fentanyl, >1000x more potent than morphine
 - Isotonitazene ~10x fentanyl potency
 - NDI 5-10x Isotonitazene potency



OBJECTIVE – APPROACH FROM ALL SIDES

Traditional forensic analysis may not be sufficient for assessing the prevalence/trends of Zenes



MARKET SURVEILLANCE
(DRUG CHECKING)



DRUG TREATMENT
PROGRAMS



POST-MORTEM
INVESTIGATIONS

METHODS – SAMPLE PREP

Toxicology Samples (drug material sample analysis is not discussed in this presentation)

- Add 1.0 mL of 0.1M Borax buffer (pH 10.4), then 3.0 mL of 70:30 N-butyl chloride/ethyl acetate
- Cap and rotate for 10 minutes
- Centrifuge at 3750 rpm for 10 minutes
- Place samples in -80 °C freezer for 15 minutes – allow the aqueous layer to freeze
- Transfer the organic layer to new clean labelled test tubes
- Add 100 µL of 10% HCl in methanol to all test tubes
- Evaporate all samples to dryness at 35°C
- Reconstitute samples with 200 µL of initial condition MPA: MPB

METHODS – INITIAL FORENSIC ANALYSIS

- Samples initially screened by LC-QTOF
- OLD INSTRUMENT:
 1. Shimadzu® Nexera XR UPLC coupled with a SciEx® Triple-TOF 5600 or X500R
 2. Phenomenex C-18 (2.7 μm , 3.0 x 100 mm)
- NEW INSTRUMENT:
 1. SciEx® ExionLC coupled with a SciEx® X500R
 2. Phenomenex C-18 (2.7 μm , 3.0 x 100 mm)



METHODS – SECONDARY ANALYSIS

- Samples confirmed by LC-QQQ
- INSTRUMENT PARAMETERS:
 1. Instrumentation: Waters® Acquity UPLC coupled with a Waters Xevo® TQ-S micro
 2. Column: Agilent Infinity Lab Poroshell C-18 (2.7 μm , 3.0 x 100 mm)

Analytes

4'-Hydroxy nitazene, Flunitazene, 5-Amino Isotonitazene, Metonitazene, N-Desethyl-Isotonitazene, Clonitazene, Etonitazene, Pronitazene, Isotonitazene, Butonitazene, Etodesnitazene



TIMELINE OF FINDINGS

- September 2022
- October 2022
- November 2022
- December 2022
- Through 2023
 - Identified in 5 additional drug material samples
 - Identified in 8 additional toxicology samples*



RESULTS - DRUG COMPOSITION

Unique ID	Glassine Bag Stamp	Quatitative Results	Qualitative Results
PDPH_2022_740	HEARSE	Fentanyl (4.6%), Xylazine (60.5%), para-Fluorofentanyl (0.5%), 4-ANPP (0.6%)	Fentanyl (1p), Xylazine (18.8p), 4-ANPP (0.3p), para-Fluorofentanyl (0.1p), Procaine (0.1p), Bromazolam (trace), Caffeine (trace), N-Desethyl Isotonitazine (trace)
PDPH_2022_741	ATCO	Fentanyl (3.5%), Xylazine (76.6%), 4-ANPP (0.4%)	Fentanyl (1p), Xylazine (28.1p), Bromazolam (0.1p), 4-ANPP (0.1p), N-Desethyl Isotonitazine (trace)
PDPH_2022_739	HEARSE	Fentanyl (5.1%), Xylazine (55.6%), para-Fluorofentanyl (0.7%), 4-ANPP (0.7%)	Fentanyl (1p), Xylazine (16.3p), 4-ANPP (0.1p), para-Fluorofentanyl (trace), N-Desethyl Isotonitazine (trace), Bromazolam (trace), Phenethyl-4-ANPP (trace)
PDPH_2022_757	SHOW AND TELL	Fentanyl (1.5%), Xylazine (61.5%), 4-ANPP (0.4%), Levamisole (3.2%)	Fentanyl (1p), Xylazine (65p), Bromazolam (0.4p), Procaine (0.2p), N-Desethyl Isotonitazene (0.1p), Flubromazepam (0.1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)
PDPH_2022_763	SHOW AND TELL	Not Quantified	Fentanyl (1p), Xylazine (5.4p), Bromazolam (0.1p), 4-ANPP (0.1p), N-Desethyl Isotonitazene (0.1p), Flubromazepam (trace)
PDPH_2022_770	N/A	Fentanyl (1.1%), Xylazine (49.3%), 4-ANPP (1%)	Fentanyl (1p), Bromazolam (1p), Xylazine (86.6p), N-Desethyl Isotonitazene (0.4p), Flubromazepam (0.1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)
PDPH_2022_781	ATCO	Fentanyl (1.9%), Xylazine (55.5%)	Fentanyl (1p), Xylazine (46.7p), Bromazolam (0.7p), N-Desethyl Isotonitazene (0.3p), 4-ANPP (0.2p), Flubromazepam (0.1p)
PDPH_2022_787	ATCO	Fentanyl (3.2%), Xylazine (49.1%), para-Fluorofentanyl (0.5%), 4-ANPP (0.5%)	Fentanyl (1p), Xylazine (21.1p), Bromazolam (0.8p), 4-ANPP (0.2p), Flubromazepam (0.2p), N-Desethyl Isotonitazene (0.04p)
BRAN_2023_0234	NA	Fentanyl (0.6%), Xylazine (0.9%), para-Fluorofentanyl (0.3%), Heroin (0.1%), Caffeine (0.4%)	Bromazolam (1p), Fentanyl (0.6p), Flubromazepam (0.1p), Fluorexetamine (0.1p), Heroin (0.1p), N-Desethyl Isotonitazene (trace), Procaine (2.4p), Xylazine (0.9p), Caffeine (0.3p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)
PDPH_2023_0021	BMX	Fentanyl (4.2%), Xylazine (60%), 4-ANPP (0.8%)	Fentanyl (1p), Bromazolam (0.4p), Flubromazepam (0.2p), N-Desethyl Isotonitazene (trace), Xylazine (17.8p), 4-ANPP (0.2p), Caffeine (0.1p)
PDPH_2023_0024	NA	Fentanyl (4.2%), Xylazine (62.6%), 4-ANPP (1%)	Fentanyl (1p), Bromazolam (0.4p), Flubromazepam (0.1p), N-Desethyl Isotonitazene (trace), Xylazine (18.8p), Caffeine (0.2p), 4-ANPP (0.2p)
PDPH_2023_0027	ILLUMINATI	Fentanyl (4.2%), Xylazine (59.6%), 4-ANPP (0.7%)	Fentanyl (1p), Bromazolam (0.4p), Flubromazepam (0.2p), N-Desethyl Isotonitazene (trace), Xylazine (16.2p), 4-ANPP (0.2p), Caffeine (0.1p)

RESULTS - FORENSIC TOXICOLOGY CASES

- Bromazolam was coincidental in the blood samples in four cases
- Flubromazepam was identified in addition to bromazolam in one case
- Maximum whole blood concentration of NDI was 5.1 ng/mL
- Few of our samples were found in combination with fentanyl
 - 100% of drug samples were combined with Fentanyl
 - Would they have even been screened for nitazene analogs if positive for fentanyl?
 - How much is underreported?



CASE STUDY

- Tablet with IP204 imprint recovered from 21-year-old decedent
- Frothy fluid found around mouth
- Fentanyl screen was negative
- Oxycodone quantified at 41 ng/mL
- Ref in Basalt report PM blood 400 ng/mL and 480 ng/mL

TEST: GC-MS Screen

SAMPLE TYPE: Urine

Collected Date/Time: 02/16/2023 09:46

Analyte:	Result:	Instrument:
COTININE	Present	GC/MS
Acetaminophen	Present	GC/MS
Oxycodone	Present	GC/MS

TEST: Confirmation/Quantitative Results

SAMPLE TYPE: Femoral Blood

Collected Date/Time: 02/16/2023 09:45

Analyte:	Result:	Instrument:
(Gr) Opioids Conf/Quant	Not detected ng/mL	
Oxycodone	41 ng/mL	
Acetaminophen	Present; <10.0 ug/mL	

SAMPLE TYPE: Urine

Collected Date/Time: 02/16/2023 09:46

Analyte:	Result:	Instrument:
THC-COOH	Present ng/mL	

CASE STUDY



Drugs.com:

Acetaminophen and Oxycodone
Hydrochloride 325 mg / 10 mg

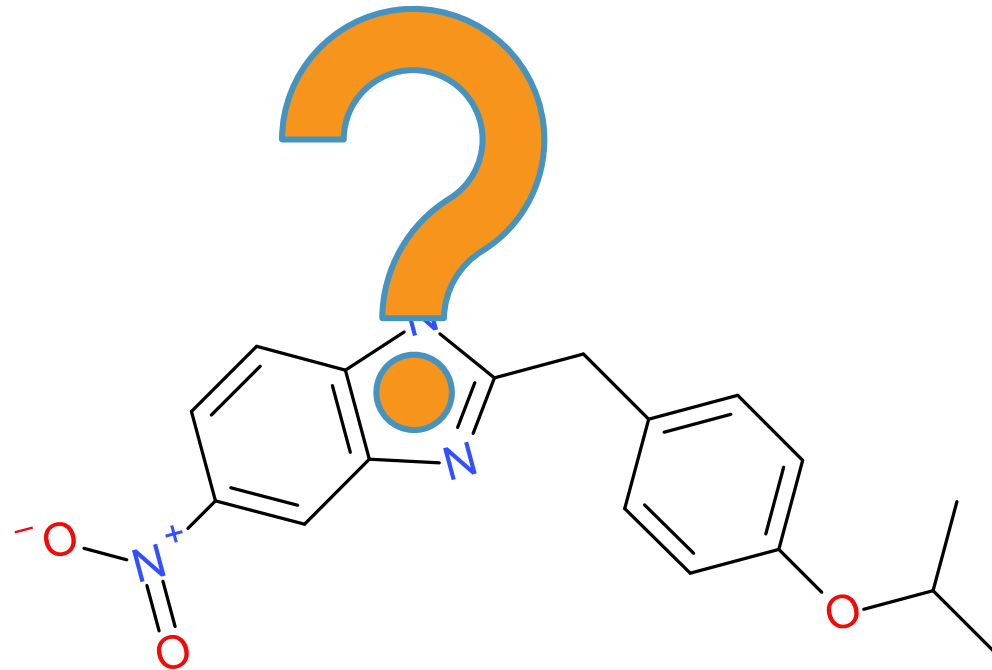
Results and Conclusions:

Exhibit #	Analyte	Concentration
1 (Blood)	<i>N</i> -Desethyl Isotonitazene	5.0 ng/mL
1	Bromazolam	Positive (<5.0 ng/mL)
1	Oxycodone	Positive
1	Acetaminophen	Positive
2 (Urine)	<i>N</i> -Desethyl Isotonitazene	1.7 ng/mL
2	Bromazolam	Positive (<5.0 ng/mL)
2	Oxycodone	Positive
2	Noroxycodone	Positive
2	Acetaminophen	Positive
3 (Pill)	<i>N</i> -Desethyl Isotonitazene	Positive
3	Bromazolam	Identified
3	Acetaminophen	Positive

DISCUSSION - OPIOID MARKET TRANSFORMATION

“If the cartels ever perfect synthesis, watch out. It’s everything bad about fentanyl but worse.”

- Like Fentanyl
 - Completely synthetic
 - High potency
- But worse
 - Much more potent
 - Can be mixed in even lower amount
- Silver lining
 - Synthesis is not accessible to cartels
 - Narcan still effective



PUBLIC HEALTH IMPACT



American Eagle models pretending to use drugs.

- Observations suggest that bromazolam was contaminated or intentionally mixed with NDI
- Individuals should be warned against purchasing chemicals online for recreational use, as the true composition is unknown
- Persons who use drugs should be aware that nitazene analogs have been identified in dope powders and illicit opioid tablets resembling pharmaceutical preparations.

CONCLUSION

- Nitazene analogs are infrequently observed and may quickly disappear from market
- Due to their potency, may avoid detection by being mixed into other DOA
- Non-targeted analysis of toxicology samples suspected of being overdoses
 - Don't stop at fentanyl (especially if concentrations are low)
 - Especially if benzodiazepines (bromazolam) are also on board.
- Common fragments/ neutral loss screening very effective!
- Current situation with NDI and other nitazene analogs is very dynamic.
 - CFSRE/NPSD hasn't seen NDI since the summer – is it gone?
 - Gone for good?
 - N-Pyrroldino-Protonitazene is the nitazene analog of the moment





Thank you!

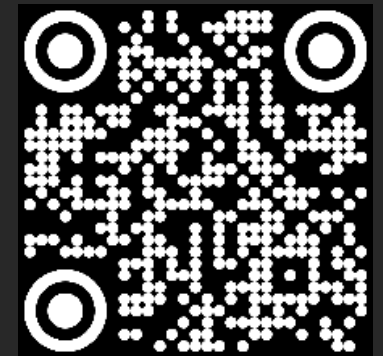
Questions?

www.cfsre.org



joshua.debord@cfsre.org

www.npsdiscovery.org



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