Monitoring Changes in the Novel Psychoactive Substance (NPS) Market through Enhanced Identification of Emerging Drugs and their Metabolites in Biological Samples

Award Number: 2015-IJ-CX-K012

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Project Purpose

Electronic dance music (EDM) festivals have become a popular venue for various types of recreational drug use, including the reported ingestion of "Ecstasy," "Molly," and/or "MDMA," which has been documented by surveys with attendees and is reflected in online discussion groups associated within the EDM culture. These terms are used interchangeably, and users are often naïve as to what active substance(s) their pills, powders, or capsules contain. Within the last four years, several adverse events associated with novel psychoactive substance (NPS) use were reported at various EDM festivals in the United States.

Both the dynamics of the synthetic drug market and diversity of NPS have resulted in analytical challenges within the forensic community in detecting and monitoring novel drug use. Currently, there is not a formalized approach to identify novel substances in toxicologically-tested populations. Novel substances often go undetected or their discovery is serendipitous. Metabolic studies for novel drugs remain limited and, generally, metabolite elucidation occurs sometime after establishing the identity of the parent compound, if at all. Using our established operational model of collecting paired specimens and self-reported drug use data from EDM festival attendees, we address some of the research needs focused on characterizing chemical compounds of forensic interest in biological systems, by providing confirmation of the parent drugs of abuse (established and emerging) and identifying metabolites in authentic specimens from a population of recreational drug users.

Project Design

Biological specimens and survey information were collected from participants at four EDM festivals in the United States over three locations during this two-year Institutional Review Board (IRB) approved study. Festival sites included Miami, FL (Spring 2016, Spring 2017), Tampa, FL (Summer 2017), and Atlanta, GA (Fall 2017). Participants were peer-recruited near

the entrance to the festival (Miami and Tampa) or within the campgrounds (Atlanta) of the festival. Participants were required to be at least 18 years old and not visibly intoxicated. Peer-recruiters began the process by explaining the purpose and significance of the study. The collection process began with the participants signing an informed consent document, which confirmed the aforementioned requirements. Blood specimens were collected into a grey-top tube. Urine specimens were self-collected in a private lavatory. Oral fluid specimens were self-collected, under recruiter supervision, according to the manufacturer's instructions using the Immunalysis Quantisal®. All samples were initially screened for abused, therapeutic, and emerging drugs, including hundreds of NPS, by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF), liquid chromatography time-of-flight mass spectrometry (LC-TOF), or gas chromatography mass spectrometry (GC-MS). All samples that screened positive for one or more drugs were sent for qualitative and/or quantitative confirmatory analysis, depending on the drug present and available confirmatory methods.

Results and Discussion

During this study, 912 biological samples (blood, urine, and oral fluid) were collected from participants at four music festivals over three geographical locations (Appendix A: Table 1). Survey responses were obtained from 691 participants during sample collection at the four festivals in 2016 and 2017, from whom at least an oral fluid sample was collected. In total, 431 males (62%) and 255 females (37%) provided survey information regarding gender, with five (1%) participants not indicating their gender. The average age of the participants in this study was 23.7 years old (±5.2 years). Across all four collection sites in 2016 and 2017, 63% of participants reported medicinal and/or recreational drug use within the past week. Marijuana was the most commonly encountered response for recent drug use, followed by Ecstasy, Molly, and/or MDMA (Appendix A: Figure 1). Figure 2 (Appendix A) shows a further breakdown of

Ecstasy, Molly, and MDMA responses. With respect to novel stimulants and MDMA, 26% (n=179) of participants reported using Ecstasy, Molly, and/or MDMA.

Miami 2016 (Blood and Urine)

Based on LC-QTOF screening results of the 13 blood samples, only two blood samples were sent for additional confirmation. No novel stimulants were detected in these blood samples. One blood sample was positive for MDMA (340 ng/mL), MDA (30 ng/mL), and amphetamine (7.7 ng/mL). The other blood sample was positive for MDMA (50 ng/mL), MDA (8.2 ng/mL), LSD (1.2 ng/mL), modafinil (0.75 ng/mL), THC (2.7 ng/mL), and THC-COOH (11 ng/mL). Survey responses paired with both blood samples indicated use of Molly.

Based on GC-MS and LC-QTOF screening results of the 50 urine samples, 38 samples were sent for respective confirmations. Novel stimulants were detected in only one urine specimen: dibutylone and butylone. The paired survey response information indicated that the participant only used marijuana. MDMA and MDA were confirmed in three individual urine samples, and amphetamine was confirmed in four urine samples. The paired survey responses with MDMA positive urine samples indicated the use of Molly (n=2) and MDMA (n=1).

Miami 2016 (Oral Fluid)

Seventy-nine oral fluid samples were positive for THC, and 61 of those samples resulted in a quantitative value (mean 77.2 [±199.1] ng/mL; median 28.7 ng/mL). Sixty-one oral fluid samples were positive for 67 common drugs of abuse (excluding THC), including samples positive for more than one substance. Fifty-seven oral fluid samples were positive for a novel stimulant and/or MDMA/MDA and were quantitatively confirmed (Appendix A: Table 2). Fourteen additional oral fluid samples were qualitatively confirmed for any remaining drugs or NPS by LC-QTOF. Of note, these results include one positive sample for 4-fluoroamphetamine (4-FA), three positives for LSD, and two positives for ketamine.

Miami 2017 (Oral Fluid)

A total of 93 oral fluid samples were positive for THC, with 83 being above the limit of quantitation (mean 104.3 [±236.1] ng/mL; median 23.3 ng/mL). Forty-six oral fluid samples were positive for 73 common drugs of abuse (excluding THC), including samples positive for more than one substance. Eighty-eight oral fluid samples were quantitatively confirmed for novel stimulants and/or MDMA/MDA by LC-MS/MS (Appendix A: Table 3), and an additional 23 oral fluid samples which were qualitatively confirmed for any remaining drugs or NPS by LC-QTOF. The NPS confirmed included 2,5-dimethoxy-4-bromophenethylamine (2C-B; n=2), 2,5-dimethoxy-4-bromoamphetamine (DOB; n=2), trifluoromethylphenylpiperazine (TFMPP; n=1), benzylpiperazine (BZP; n=1), LSD (n=2), ketamine (n=3), and etizolam (n=1).

Tampa 2017 (Oral Fluid)

THC was confirmed in 55 of the 131 oral fluid samples by LC-MS/MS with 48 samples being positive above the LOQ (mean 109.2 [±238.2] ng/mL; median 32.2 ng/mL). Nine oral fluid samples were positive for 28 common drugs of abuse (excluding THC), including samples positive for more than one substance. Thirty-three oral fluid samples were quantitatively confirmed for novel stimulants and/or MDMA/MDA (Appendix A: Table 4). Twenty additional oral fluid samples were qualitatively confirmed for any remaining drugs or NPS by LC-QTOF, including three positive samples for LSD, two for ketamine, and one for psilocin.

Atlanta 2017 (Oral Fluid)

One hundred and five oral fluid samples were confirmed for THC, with 86 samples being above the LOQ (mean 97.3(±159.1) ng/mL; median 29.3 ng/mL). Fifty-nine oral fluid samples were positive for 142 common drugs of abuse (excluding THC), including samples positive for more than one substance. Eighty-three oral fluid samples were quantitatively confirmed for novel stimulants and/or MDMA/MDA (Appendix A: Table 5). Twenty-seven additional oral fluid

samples were qualitatively confirmed for any remaining drugs or NPS by LC-QTOF, including positive samples for LSD (n=14), ketamine (n=8), and methylenedioxyethamphetamine (MDEA; n=2).

Temporal Trends - Miami

Since oral fluid samples were collected in Miami from 2014 to 2017 (n=1,233), this allowed the comparison of novel stimulant positivity, as well as MDMA positivity, over time at this location. Figure 3 (Appendix A) shows the change in novel stimulant positivity with respect to year. The data is shown as percent positivity in terms of only novel stimulants, MDMA, and MDA. While many of the novel stimulants remain low in positivity, it is important to note the disappearance of alpha-PVP, the rapid rise and decline of ethylone, and the emergence of dibutylone and N-ethyl pentylone.

Geographic Trends - 2017

Since oral fluid samples were collected in three locations (Miami, Tampa, and Atlanta) in 2017, this allowed for the comparison of novel stimulant positivity, as well as MDMA positivity, across these geographic locations. Figure 4 (Appendix A) shows the change in novel stimulant positivity with respect to location. The data is shown as percent positivity in terms of only novel stimulants, MDMA, and MDA. 4-FA, alpha-PVP, and pentylone were excluded due to no positivity or positivity only at one location. Based on this comparison, there does not seem to be an apparent distinction with relation to novel stimulant positivity and location (<5% difference), although N-ethylone percent positivity was highest in Tampa, and MDMA percent positivity was lowest in Tampa.

Novel Stimulant vs. Survey Response – All Years

Following analytical confirmation of novel stimulants, MDMA, and MDA, the self-reported survey data responses of Ecstasy, Molly, or MDMA for drug ingested were compared to

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the confirmatory findings. Figure 5 (Appendix A) shows the relationship between Ecstasy, Molly, and MDMA responses vs. analytical findings of novel stimulants and/or MDMA and MDA. These data show that MDMA was detected in the majority of samples (>50%), regardless of the terminology used by the participant. It is interesting to note that no response for Ecstasy was paired with a novel stimulant detection only, and all positive samples contained at least MDMA. This leads to the possible conclusion that users purchasing Ecstasy are more likely to obtain preparations containing MDMA; however, it is also possible that these preparations may contain additional novel stimulants.

Metabolite Identification

Analysis of data from LC-QTOF analysis of the human liver microsome (HLM) incubation mixtures resulted in the identification of five metabolites of dibutylone, all of which were identified in authentic specimens. Four metabolites of N-ethyl pentylone were identified via HLM incubations, all of which were also found in authentic specimens. Proposed metabolic pathways and further details related to the in vivo identifications can be found in the resulting publications (1,2).

Conclusions

This research study sought to use a cohort of EDM festival attendees as a sentinel population to monitor changing patterns and regional trends of NPS use in the United States. In order to accomplish this goal, the strategy was to: 1) collect biological specimens and accompanying survey information from festival attendees; 2) comprehensively analyze all specimens for the accurate detection of common drugs of abuse and emerging drugs; 3) generate and identify metabolites of emerging drugs in biological specimens collected; 4) tabulate and analyze all data from this study and previously collected data, creating a four year time period across three locations to monitor trends and identify any pertinent information that would be

beneficial to the forensic science communities; and 5) disseminate the results from this study within the forensic science communities for increased knowledge and awareness surrounding this population, the emergence and turnover of NPS, and the biomarkers useful in distinguishing recent use of these drugs. All of these objectives directly relate to the furthering of information relating to investigations of criminal activity, drug use and possession, impaired driving, drug-facilitated sexual assault, and other drug-related crimes.

Through sample analysis, we were able to identify dibutylone and N-ethyl pentylone for the first time within this population based on our data, and identify their respective biomarkers following extensive metabolic studies. The variation of novel stimulants increased from year to year. Only four novel stimulants were detected in 2014 compared to six novel stimulants in 2017. Drugs initially popular in 2014, like alpha-PVP, were not detected in any specimens collected during this two-year period, following scheduling of its precursors in China in October 2015. Novel stimulants identified included methylone, dimethylone, ethylone, butylone, dibutylone, pentylone, eutylone, N-ethyl pentylone, and 4-FA, in addition to the common amphetamines MDMA and MDA.

Of the 1,233 oral fluid specimens collected in our study, 352 (28.5%) confirmed positive for a novel stimulant, MDMA, and/or MDA. Compared to all oral fluid samples that were positive for at least one or more drug of abuse and/or NPS (n=684), the positivity rate for a novel stimulant, MDMA, and/or MDA was 51.5%. The majority of oral fluid samples collected contained more than one drug or NPS, suggesting high rates of poly-drug use within this population, possibly increasing the potential for adverse events.

The initial hypothesis regarding novel stimulant positivity among different geographical locations was that there would be a difference in the novel stimulants seen because of drug

trafficking patterns or regional variability. A limitation of the geographical comparison is that all locations are located in the southeastern part of the United States. There was not a distinct difference in positivity over locations from Miami to Tampa to Atlanta. While there are slight differences in positivity between Miami and Atlanta compared to Tampa, this could not be determined to be indicative of or related solely to the geographic location. N-ethyl pentylone positivity was decreased in Atlanta (September 2017) compared to Tampa (May 2017), which could either reflect regional differences or a shift back to MDMA. MDMA and MDA positivity were both relatively stable across all three locations. Interestingly, the results from Miami and Atlanta are more consistent, the two locations farthest from each other, rather than with Tampa, the location in the center. City and attendee demographics could play a role in this distinction, but again this information cannot be discerned from this study.

Following their initial identification in this population, the metabolic profiles of dibutylone and N-ethyl pentylone were studied. During this research period, we were able to confirm the metabolism of dibutylone to butylone for the first time, as well as identify a selective biomarker for toxicological testing: 1-(1,3-benzodioxol-5-yl)-2-(dimethylamino)butan-1-ol (hydrogenation of the ketone on dibutylone). The metabolic profiling of N-ethyl pentylone resulted in the identification of four metabolites. Consistent with dibutylone, the most prominent metabolite of N-ethyl pentylone was found to be 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-ol (hydrogenation of the ketone on dibutylone).

Implications for Policy and Practice

The goals of this project were to further develop techniques for and demonstrate the value of monitoring of an at-risk, high incidence NPS-using population as a sentinel group for tracking changes in the NPS market in the United States. There are several lessons learned from the

project, in both the public health and public safety domains, where the lessons learned from this study can influence policy.

Drug use has significant public health implications, including immediate life-threatening, psychological, and physical effects ranging from effects on mood, cognition, appetite, wakefulness, hypertension, respiration, and cardiac effects including stroke and cardiac arrest. Drug use also may affect the individual's academic, mental, social, and economic health. The consequences from these effects in turn impact social and health systems and impact public costs. Crossing many pharmacological categories including stimulants, hallucinogens, narcotics, depressants, and dissociatives with a wide range of physiological and psychological side effects, NPS drugs have the potential to negatively impact the economic health and social stability of many social classes in the United States.

Implications for Further Research

We have developed a novel, robust, efficient, and evidence-based model for monitoring drug use in at-risk populations. The lessons learned were in areas including logistics of deployment including field sample collection, specimen storage and shipping, successful recruiting strategies for encouraging participation by subjects, coordination with local law enforcement, interview instruments for collecting self-report drug use data, addressing human subjects concerns of IRBs, identification of the most efficient sample collection techniques, validation of analytical methods for comprehensive screening of traditional recreational, therapeutic, and NPS drugs, and dissemination channels for sharing the information. This model can be readily deployed to study other drugs or other drug-using populations.

With respect to NPS use in the EDM population, additional follow-up testing of the population is strongly recommended, as the latest data shows that the use profile of available and

popular NPS drugs is still changing rapidly and MDMA positivity is increasing. Future studies could be conducted more efficiently with fewer peer recruiters using an oral fluid-only model.

Opportunities for future research in this area include the following: continued updating and distribution of libraries of NPS drugs and their metabolites, continued monitoring of the EDM population, expanding the number of events tested to broader geographic locations and different music genres, performing more comprehensive surveys using smartphone technology, performing on-site testing of drug preparations and doses using mobile GC-MS technology, and performing testing of contents of amnesty bins.

Acknowledgements

The authors of this manuscript would like to acknowledge the following for their contributions to this project: Jill Yeakel (Lehigh Valley Toxicology), Donna Papsun (NMS Labs), Francis Diamond (NMS Labs), the Armed Forces Medical Examiner's Office: Division of Forensic Toxicology, staff at the Fredric Rieders Family Foundation, and graduate students from Florida International University, Arcadia University, and the University of South Florida.

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2. Alex J. Krotulski, Amanda L.A. Mohr, Donna M. Papsun, and Barry K. Logan. (2017) Dibutylone (bk-DMBDB): Intoxications, Quantitative Confirmations, and Metabolism in Authentic Biological Specimens. *Journal of Analytical Toxicology*. [In press].

Appendix A

Table 1: Biological samples collected at all festivals (2016-2017)

Festival	Year	Blood Samples	Urine Samples	Oral Fluid Samples
Miami	2016	13	50	244*
Miami	2017	-	-	308
Tampa	2017	-	-	131
Atlanta	2017	-	-	166

**Includes 158 oral fluid samples from the crowd*
(-): Sample type not collected

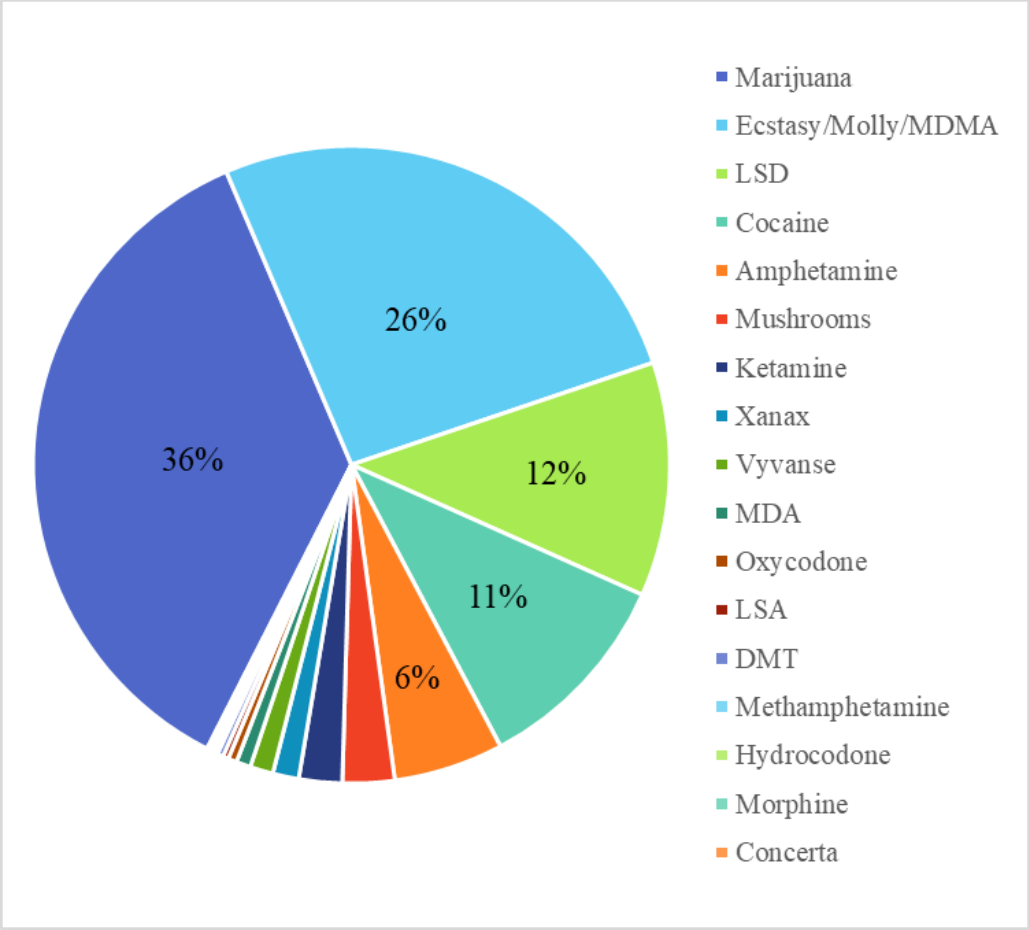

Figure 1: Self-reported medicinal and/or recreational drug use over four sample collection sites (2016-2017).

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Appendix A

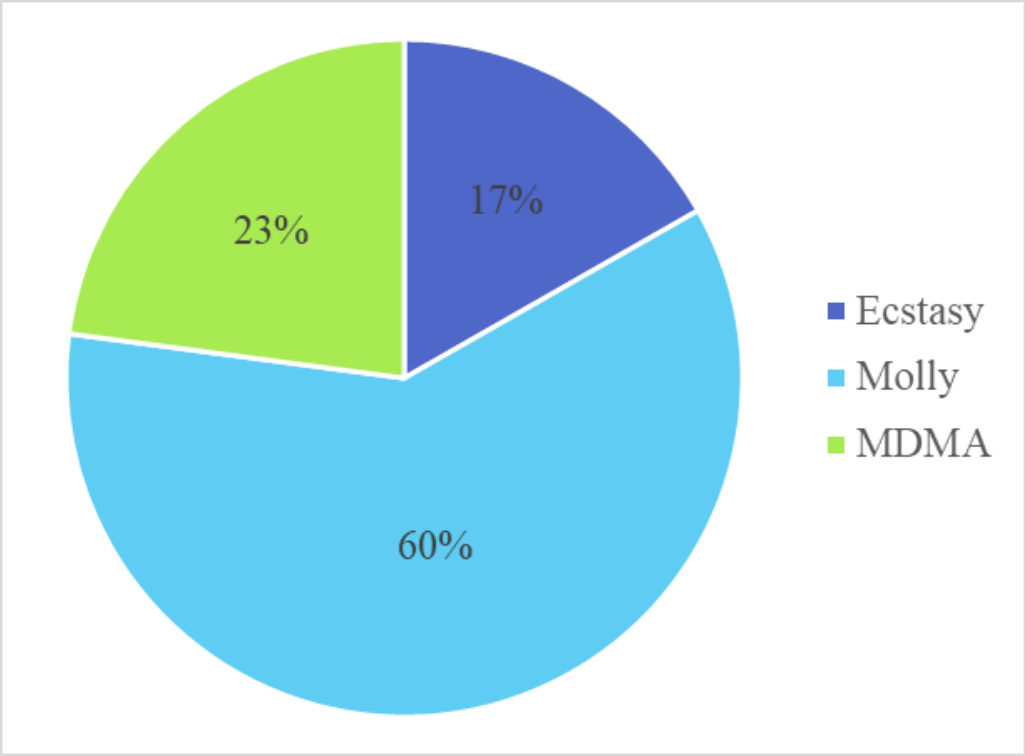

Figure 2: Self-reported Ecstasy, Molly, and MDMA use over four sample collection sites (2016-2017).

Table 2: Confirmatory results for novel stimulants in oral fluid samples* (Miami 2016)

Analyte	Positive Samples	Samples with Quant. Conc.	Average Conc. (±Std. Dev.) (ng/mL)	Median (ng/mL)	Range (ng/mL)
Methylone	3	0	-	-	-
Dimethylone	3	1	4.7	-	-
Ethylone	18	4	27.9 (±25.3)	26.7	4.4 - 53.9
Butylone	12	4	568.7 (±803.0)	248.7	16.6 - 1761
Dibutylone	12	3	728.9 (±1036)	137.6	123.2 - 1926
Eutylone	0	-	-	-	-
Pentylone	1	0	-	-	-
N-ethyl pentylone	0	-	-	-	-
MDA	36	24	508.1 (±2028)	24.0	6.2 - >10000
MDMA	43	32	561.9 (±927.7)	236.1	4.1 - 4298

*Some oral fluid samples were positive for more than one drug

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Appendix A

Table 3: Confirmatory results for novel stimulants in oral fluid samples* (Miami 2017)

Analyte	Positive Samples	Samples with Quant. Conc.	Average Conc. (±Std. Dev.) (ng/mL)	Median (ng/mL)	Range (ng/mL)
Methylone	4	0	-	-	-
Dimethylone	1	0	-	-	-
Ethylone	3	1	197.5*	-	-
Butylone	6	5	160.8 (±310.9)	9.9	4.6 - 715.0
Dibutylone	11	4	53.1 (±67.0)	26.3	7.3 - 152.7
Eutylone	0	-	-	-	-
Pentylone	1	0	-	-	-
N-ethyl pentylone	3	1	8.7*	-	-
MDA	70	54	71.4 (±124.1)	29.0	4.1 - 657.5
MDMA	76	72	773.0 (±1797)	213.3	4.4 - >10000

*Some oral fluid samples were positive for more than one drug

Table 4: Confirmatory results for novel stimulants in oral fluid samples* (Tampa 2017)

Analyte	Positive Samples	Samples with Quant. Conc.	Average Conc. (±Std. Dev.) (ng/mL)	Median (ng/mL)	Range (ng/mL)
Methylone	2	0	-	-	-
Dimethylone	0	-	-	-	-
Ethylone	2	0	-	-	-
Butylone	0	-	-	-	-
Dibutylone	1	0	-	-	-
Eutylone	0	-	-	-	-
Pentylone	0	-	-	-	-
N-ethyl pentylone	11	5	317.8 (±593.9)	35.2	12.6 - 1377
MDA	24	19	48.4 (±93.2)	16.2	4.8 - 412.5
MDMA	26	24	1419 (±3017)	132.4	8.0 - >10000

*Some oral fluid samples were positive for more than one drug

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Appendix A

Table 5: Confirmatory results for novel stimulants in oral fluid samples* (Atlanta 2017)

Analyte	Positive Samples	Samples with Quant. Conc.	Average Conc. (±Std. Dev.) (ng/mL)	Median (ng/mL)	Range (ng/mL)
Methylone	2	0	-	-	-
Dimethylone	1	0	-	-	-
Ethylone	2	1	72.8	-	-
Butylone	3	3	270.4 (±242.1)	208.5	65.2 - 537.5
Dibutylone	3	3	773.9 (±676.0)	652.5	166.9 - 1502
Eutylone	1	0	-	-	-
Pentylone	0	-	-	-	-
N-ethyl pentylone	8	3	60.3 (±84.9)	12.5	10.2 - 158.4
MDA	71	49	33.4 (±35.2)	23.8	4.1 - 171.1
MDMA	79	75	663.9 (±1772)	171.2	4.0 - >10000

*Some oral fluid samples were positive for more than one drug

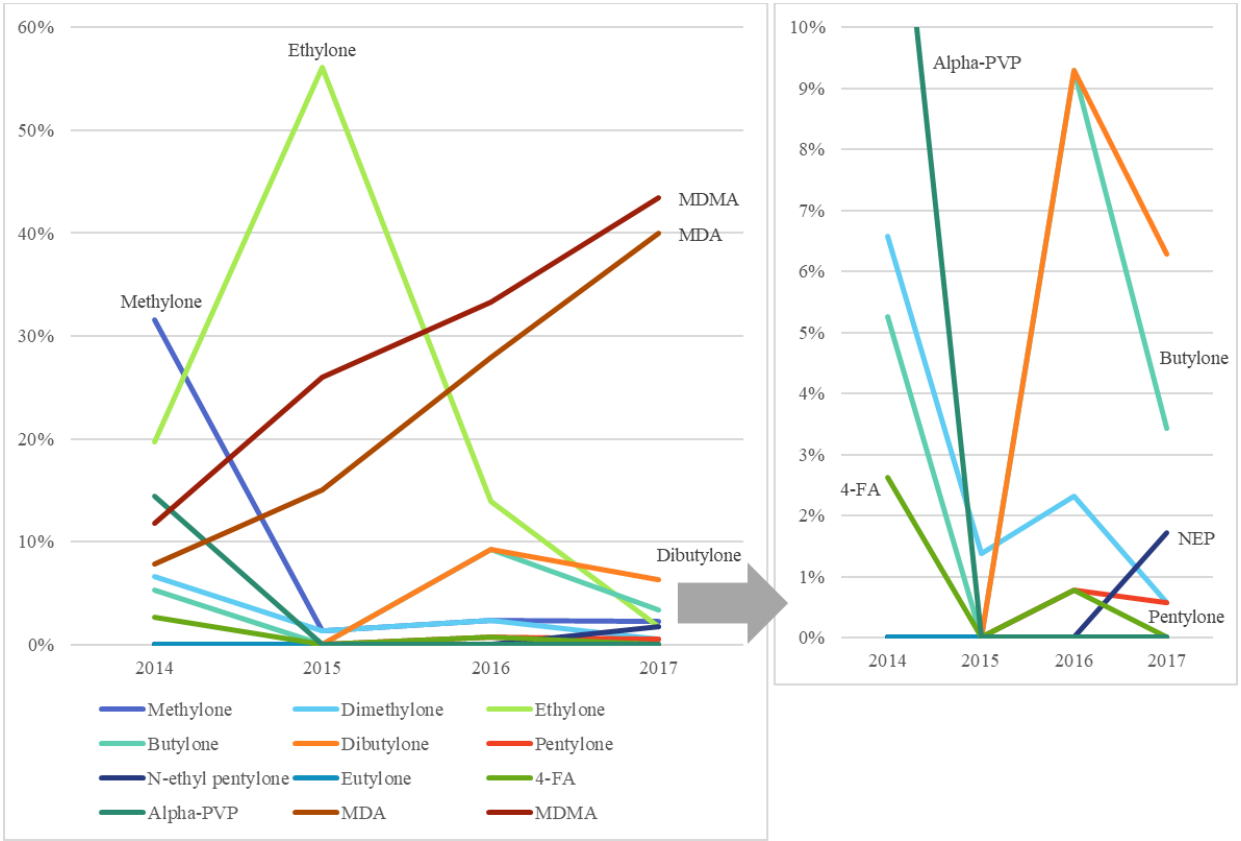

Figure 3: Novel stimulant percent positivity across four years (Miami only)

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Appendix A

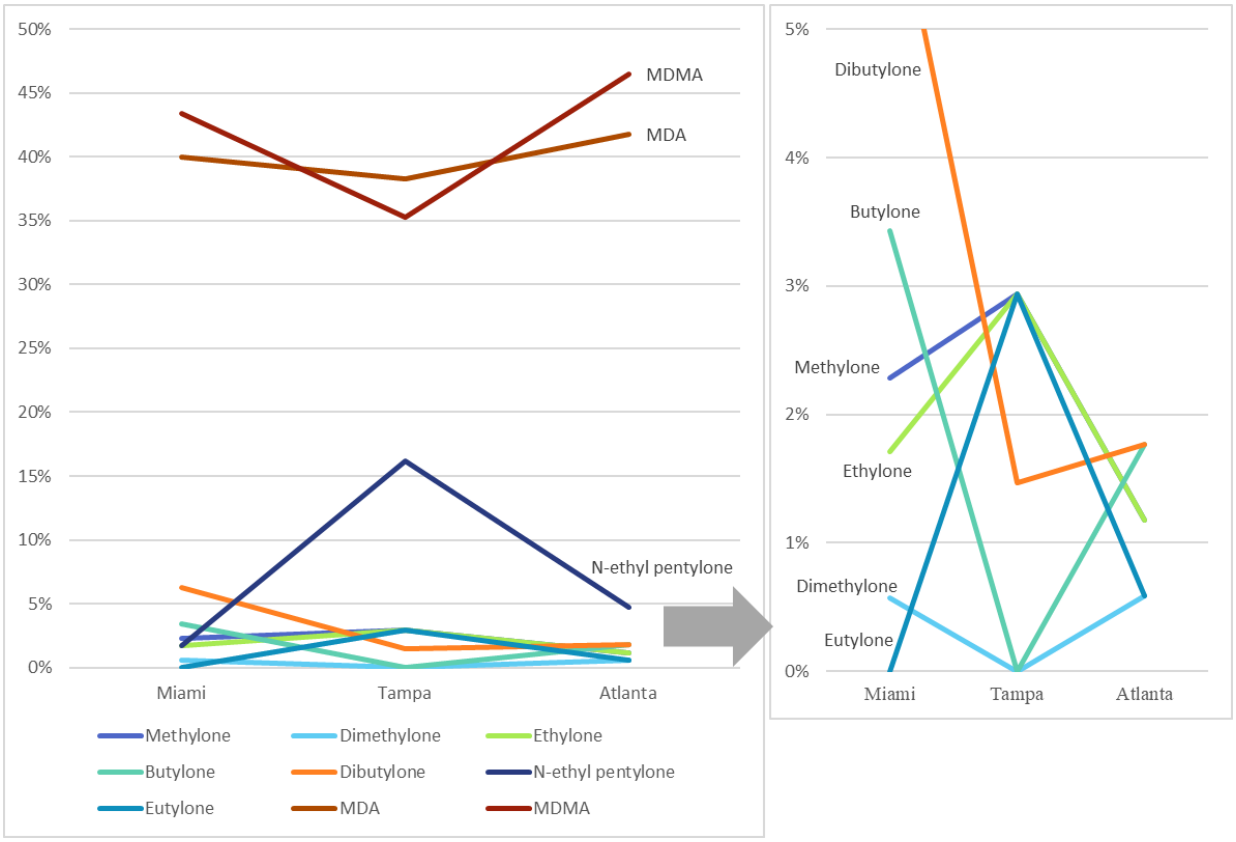

Figure 4: Novel stimulant percent positivity across three locations (2017 only)

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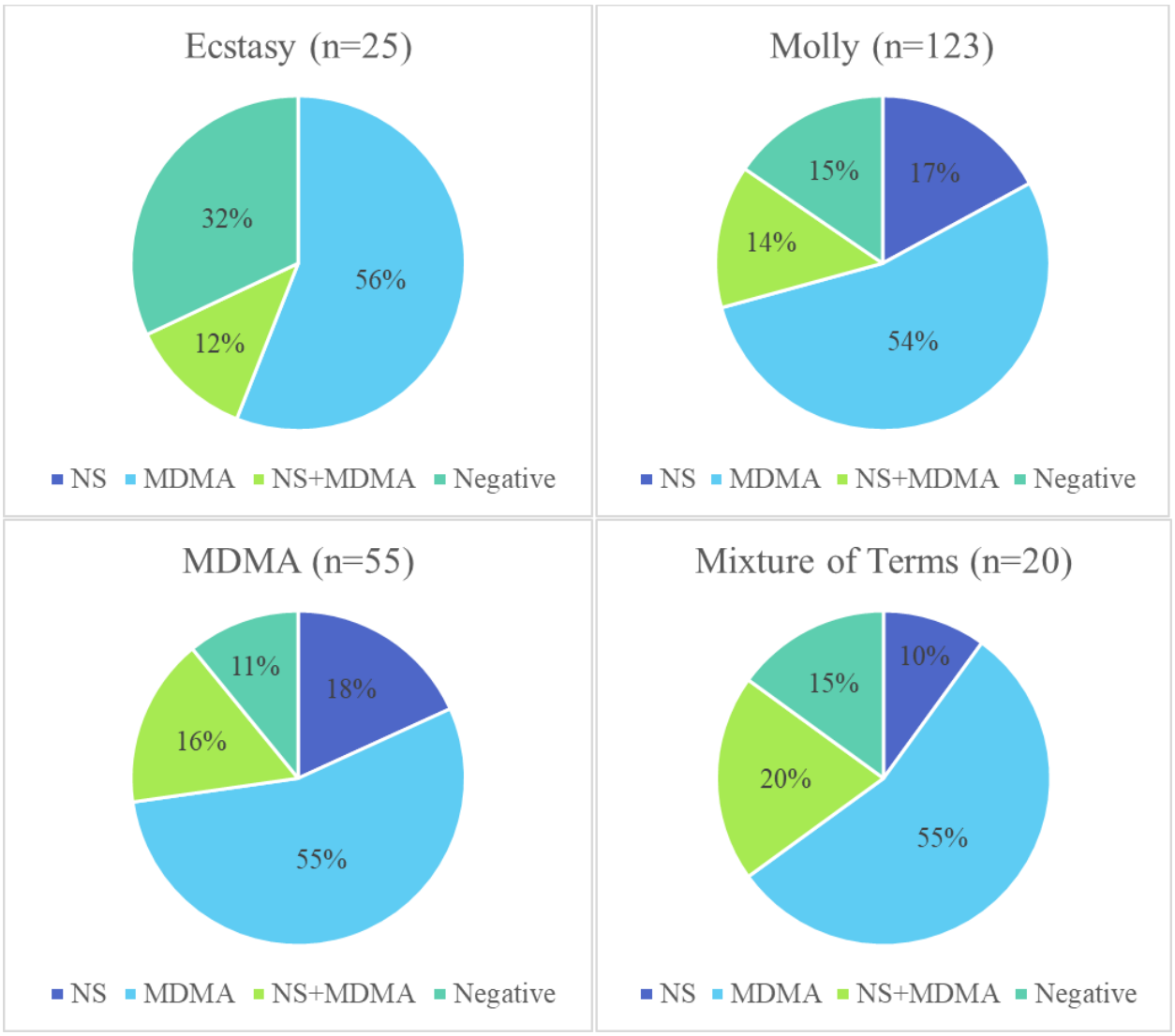

Figure 5: Overall Ecstasy, Molly, and MDMA survey responses vs. novel stimulants positivity