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► To cite this version:

Camille Richeval, Véronique Dumestre-Toulet, Jean-François Wiart, Xavier Vanhoye, Luc Humbert, et al.. New psychoactive substances in oral fluid of drivers around a music festival in south-west France in 2017. Forensic Science International, 2019, 297, pp.265 - 269. <10.1016/j.forsciint.2019.02.029>. <hal-03486829>

HAL Id: hal-03486829

<https://hal.science/hal-03486829v1>

Submitted on 20 Dec 2021

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New Psychoactive Substances in oral fluid of drivers around a music festival in south-west France in 2017

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ABSTRACT

Driving under the influence of drugs (DUID) is a worldwide problem with potentially major forensic and life-threatening consequences. Although it is obvious that **new psychoactive substances (NPS)** could lead to impaired driving, the prevalence of NPS use in a DUID context is unknown as the applied roadside screening tests for drugs of abuse (DOA) are not adapted for NPS detection. This work aims to test oral fluid (OF) specimens for NPS in French drivers circulating around two music festivals (Artsenik 2017 and Garorock 2017) in order to assess the prevalence of consumption and the kind of used NPS in this particular population. OF samples consisted in dried saliva spots obtained from used Drugwipe-5S® tests (after a positive or negative roadside screening test for DOA). These OF were analyzed using a liquid chromatography coupled with tandem mass spectrometry or high-resolution mass spectrometry method. NPS were detected in 17 out of the 229 OF collected specimens (7.4%). Eleven various NPS were identified (number of identification): 5F-AKB48 (2), MAM2201 (1), JWH122 (1), 4F-PVP (1), 3- or 4-MMC (2), fluoromethamphetamine (1), ketamine (3), MXE (3), methoxyketamine (1), 6-APB (2) and 25C-NBOMe (1). There is an apparent effect of the music festival proximity on the prevalence of NPS in OF from this controlled driver population compared to that of 140 controlled drivers from Northern France analyzed in the same period (7.4% *versus* 3%). The variety of used NPS **appears to be increasing** (e.g. large proportion of cyclohexanones). In addition, 5% of drivers initially roadside-tested negative for DOA were in fact **driving after NPS use** in this specific population. From a forensic perspective, these results confirm the reality of driving after NPS use in French drivers, **notably in those driving to or from a music festival.**

KEYWORDS: New psychoactive substances, drivers, music festival, forensic oral fluid, LC-HRMS, LC-MS/MS

Introduction

New psychoactive substances (NPS) are new narcotic or psychotropic drugs which are not controlled by the 1961 United Nations Single Convention on Narcotic Drugs or the 1971 United Nations Convention on Psychotropic Substances [1]. NPS concern substances that usually (but not exclusively) replicate chemical structures and/or pharmacological effects of classical drugs of abuse (DOA). The use and online availability of NPS has rapidly increased over the last decade. For instance, 51 new substances were detected for the first time in European countries in 2017 (the maximum of NPS detected for the first time in Europe in particular year was 101 in 2014) and the number of new substances monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was more than 670 by the end of 2017 [2]. NPS are challenging all over the world in several respects: e.g. international and European drug policies to respond as efficiently as possible to the increasing and dynamic NPS market with regard to human health, analytical testing difficulties for detection of NPS in biological samples in clinical and forensic cases including intoxications or driving under the influence of drugs (DUID) [3-6]. In this last way, recent knowledge on NPS toxicodynamics demonstrates that these compounds have potential effects on driving ability: e.g. dissociative drugs can lead to detachment with distortions of space, time and body image which are inconsistent with safety driving [7,8]. Therefore, several DUID cases report mental confusion and/or agitation in individuals positive for these compounds [9-19]. These cases are observed after additional analytical screening procedures performed in order to identify possible NPS in cases of accidents when initial standard DOA assays revealed negative results. From a forensic perspective, the main pitfall is the NPS analytical detection owing to high frequency of new products, low concentrations in biological samples, and metabolism. Indeed, detection of driving under the influence of NPS is particularly challenging, as the currently applied roadside screening tests are not adapted for NPS detection. Indeed, action against DUID often starts with an on-site immunological screening focusing on “classical” DOA: i.e. cannabis, cocaine, amphetamine derivatives and heroin use. In France, roadside testing is currently performed in oral fluid (OF) using an on-site immunological screening for DOA (Drugwipe[®] 5S from Securetec). Such rapid tests are not available for NPS. In the case of a positive result of this roadside test for DOA, a blood sample or an OF sample (since June 2017 in France) is subsequently collected for confirmation step using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). In 2016, we tested French drivers’ OF samples for NPS: these samples consisted in dried saliva spots (DSS) collected from used Drugwipe-5S[®] tests (after a positive

or negative roadside screening test). These roadside immunochemical tests for DOA were performed in drivers' OF after unannounced roadside tests (mainly), traffic offences, minor or fatal road accidents. Between January and December 2016, a total of 101 DSS from Drugwipe-5S[®] tests negative ("Neg") for DOA, was collected in one area from North of France, Lille. Three out the 101 collected DSS (3%) were positive for NPS [20]. In 2017, the same study was repeated: four (3%) out the 140 DSS collected (68 "Pos" and 72 "Neg" for DOA) in North of France were positive for NPS [21].

In the same year, we tested OF specimens for NPS in French drivers circulating on a short time period (few days) around two music festivals (Artsenik 2017 and Garorock 2017) in order to assess the prevalence of consumption and the kind of used NPS in this particular population of drivers **submitted to roadside screening test for DOA**.

Material and methods

OF samples collection

The two music festivals took place in South-west France. The Art-Scenyk festival was held in late June 2017 at La Reole (Gironde, France, 24-26th June 2017) and was an electronic music festival: Techno, Acid, Electro, Hardcore etc... The Garorock Festival was held in early July at Marmande (Lot-et-Garonne, 29th June- 3rd July 2017) and was a more general-interest style of music and a more important festival (over 100,000 participants). Roadside checks were organized around the two festivals: e.g. the police officers were divided into 7 control zones around the Garorock festival. Random checks were unannounced: generally, checks and subsequently on-site screening for DOA were decided by police officer owing to suspicious attitude (e.g. driver's reaction for hesitation or other signs that he's hiding something). Standard roadside testing for DOA was performed in oral fluid (OF) using an on-site immunological screening device (Drugwipe[®] 5S from Securetec). Anonymized Drugwipe-5S[®] tests (after a positive or negative roadside screening test) were collected by police officers.

Analytical method

DSS collected from used Drugwipe-5S[®] tests were analyzed using a previously published reported method [20-22]. The used Drugwipe-5S screening tests were broken down and two of the three OF collection pads (DSS) were analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and high resolution mass spectrometry (LC-HRMS), and database containing more than 1400 compounds including 550 NPS and their metabolites (at the analysis period, end of 2017). These assay results in DSS consisted in a

qualitative response (presence: yes or no) and concentrations of NPS in OF were not assessed. Briefly, the two pads were soaked in 300 µL of methanol for an hour at room temperature in order to extract NPS putatively present in DSS. After centrifugation, 50 µL of internal standard solution (methyl-clonazepam and β-OH-ethyltheophylline) were added to 100 µL of the obtained supernatant. The methanolic solution was then evaporated, reconstituted with 100 µL of a mixture (80:20, v:v) of methanol/formic acid 0.1 % and ammonium formate buffer 5 mM at pH 3, and 10 µL of this extract were analysed using both LC-MS/MS and LC-HRMS. NPS detection was considered as positive when the obtained chromatographic peak exhibits a signal-to-noise ratio of at least 3, and agrees with usual mass spectrometry specificity guidelines (e.g. expected relative retention time, presence of two product ion transitions with an expected ratio for MS/MS, and expected accurate mass and isotopic pattern for HRMS).

Results

229 DW were collected: 9 around the Art-Scenyk Festival and 220 around the Garorock Festival. For this second festival, we were able to distinguish drivers that went out or came in the area of the festival. Among these 229 collected DW, 133 were initially negative for salivary screening for DOA (“Neg”) and 96 positives at least for one DOA (“Pos”).

NPS were detected in 17 out of the 229 OF (DSS) collected specimens (7.4%) which was or not associated with a classical DOA. **NPS were the only compounds detected in 7 out of the 17 OF with NPS.** Eleven various NPS (18 identifications) were identified in this 17 OF samples (number of identification): 5F-AKB48 (2), MAM2201 (1), JWH122 (1), 4F-PVP (1), 3- or 4-MMC (2), fluoromethamphetamine (1), ketamine* (3), MXE (3), methoxyketamine (1), **5- or 6-APB (2) and 25C-NBOMe (1)** (Figure 1 and Table 1) [***ketamine was included in these results because its intake can impaired driving ability and ketamine is not detected by standard roadside testing**]. **DOA were also detected in 10 out of the 17 OF positive for NPS (Figure 1): THC, MDMA (together with MDA and amphetamine), and cocaine.** It is of note that 5% of drivers initially roadside-tested negative for DOA (7 out the 133 “Neg”) were positive for NPS in OF (4F-PVP, MXE, 6-APB, 25C-NBOMe).

Discussion

Several studies have shown a higher frequency of the presence of psychoactive drugs in injured or killed drivers compared to drivers in general traffic. Generally, connection between driving under the influence of drugs and traffic accidents is demonstrated, but it does not

systematically implicate drug use as the main reason for the accidents. In drivers under suspicion of driving under the influence psychoactive substances, alcohol remains the most frequent found compound in France as well as in EU, followed by DOA (in descending order: THC, cocaine, and amphetamine), while NPS are seldom detected [23].

Trend data on NPS prevalence are limited. Worldwide, the NPS prevalence in the general population is estimated to be fairly low (0.1 % for 15-64 years old in 2013). In Europe, 3% past-year prevalence of use of NPS among young adults (aged 15-24 years) was reported in 2014 [24]. NPS abuse seems more elevated in high-risk groups as, for example, in Australia where 40% of regular psychostimulant users had used a NPS [25]. Nevertheless, a general problem in understanding NPS use is that users often do not know which substance they have taken or which substance(s) the product they consume really contains. That may partly explain why some surveys show an increase while others show stable or decreasing trends of NPS use [26]. Only very few data were published in drivers populations. Simonsen et al. reported NPS occurrence based on blood analysis from 3284 Danish drivers suspected of impairment and drivers stopped at random to test for impairment in 2015 and 2016: only six cases (0,18%) were found positive for NPS (LSD, alpha-PVP, mCPP, delorazepam) [23]. From January to August 2015, we have screened for NPS in 558 blood samples obtained on roadside controls in Belgium (Mons, Turnhout, Mechelen) after a positive Drugwipe 5S[®] test: the positivity rate for NPS in these blood samples was 7% (37 cases) [22]. These studies are limited by the fact that only drivers roadside detected positive (using immunochemical test) for DOA are involved. Using the same method than in the present work, we tested French drivers' OF samples for NPS in 2016: 3 out (3%) the 101 DSS from Drugwipe-5S[®] (initially tested "Neg" for DOA, and collected from North of France, Lille) were positive for NPS (only cathinone derivatives were found in these 3 OF: ethylone, methylone, and MDPV) [20]. In 2017, the same study was repeated: 4 (3%) out the 140 collected DSS (68 initially tested "Pos" and 72 "Neg" for DOA) in the same area from North of France were positive for NPS (4-methyl-NEP, methamnetamine, LSD and ethylphenidate) [21]. The present work took place over the same period, but in South-west France and around a music festival: DSS tested positive for NPS appear clearly more (7.4% versus 3%). This increase of prevalence reflects probably the drug use at the music festivals.

Indeed, the NPS use prevalence appears higher in specific populations of dance clubs or music festivals than in general population. The USA Global Drug Survey, an annual international survey on drug use, found that in 2011/2012, compared to respondents reporting no nightclub attendance in the last year, those who reported attending (vs. those not attending)

reported higher prevalence of use of mephedrone, 2C-B, 2C-I, MXE, N,N-dimethyltryptamine, and synthetic cannabinoids [27]. NPS use (mainly synthetic cannabinoids) was endorsed by 39.8% (all of whom identified as being experienced DOA users) of 171 attendees of 2 music festivals in Colorado in 2015 and 2016 [28]. In 2015, 35.1% of adults attendees of electronic dance music parties at nightclubs and festivals in New York City reported lifetime use of any NPS. Self-reported use of synthetic cannabinoids was most prevalent, followed by phenethylamines, synthetic cathinones, other psychedelics, tryptamines, and dissociatives [29]. Attitudes and beliefs about NPS and how they relate to self-reported use were examined in this electronic dance music population (1,048 individuals, New York City, USA, 2016). Less than half the sample reported being familiar with NPS other than “bath salts” and synthetic cannabinoids. Cited NPS were dissociative NPS (e.g., methoxetamine), 2C drugs (e.g., 2CI), tryptamines (e.g., 4-MeO-DMT), and NBOMe (e.g., 25i-NBOMe) [30,31]. In Germany (Munich, 2016), 1571 questionnaires were used in order to explore the 12-month prevalence and type of NPS consumption in patrons of electronic music events population: a 12-month prevalence of 21.2% was reported for ketamine, and of 20% for other NPS (bath salts, smoking mixtures and research chemicals) in this specific population [32]. In a cross-sectional study, 44 pooled urine samples were collected (from three urinals) at the largest Danish music festival in 2016: 77 drugs and metabolites were qualitatively identified including ketamine and methylphenidate [33]. In New York City, hair samples of drug users of the electronic dance music scene (New York City, summer 2016) were collected, and subsequently tested (using LC-MS/MS) for drugs. Three quarters of these 90 hair samples tested positive for MDMA and 33% for an NPS: mainly synthetic cathinones (e.g., butylone, ethylone, pentylone, methylone, alpha-PVP), other NPS stimulants (e.g., 4-FA, 6-APB), or new dissociatives (e.g., methoxetamine, diphenidine) [34,35]. Oral fluid analysis was applied in 4-year study (2014-2017) in order to assess NPS use in music festivals: 1,223 oral fluid specimens were collected from participants attending large multi-day electronic dance music festivals (in Miami, FL; Tampa, FL; and Atlanta, GA, USA). This study reported a total of 221 detections of NPS, and detected NPS were methylone, dimethylone, ethylone, butylone, dibutylone, eutylone, pentylone, N-ethyl pentylone, ephylone, alpha-PVP and 4-fluoroamphetamine [36,37]. Overall, NPS used in music events encompass cathinones, synthetic cannabinoids, psychedelic phenethylamines and (even more in Europe) dissociative arylcyclohexylamines. Some studies examined medical problems that are frequently encountered during electronic dance music events. For instance, in Belgium, 106 blood samples from intoxicated patients during two editions (2013 and 2014) of an indoor

electronic dance event with around 30,000 attendants were included in a toxicology study. Additional specific analyses for NPS were performed for 20 cases: the psychedelic phenethylamines 25B-NBOMe and 25C-NBOMe were found in blood sample of one agitated patient [38]. It is of note that a similar clinical study was conducted at the Garorock Festival in 2017. Dried blood spots, urine and hair samples from 25 intoxicated patients were collected at first aid spots in order to determine the psychoactive substances involved in the more serious intoxication observed cases. Toxicological screening results were dominated by MDMA and cocaine positive results, and no NPS was detected [39]. These various data about NPS use in populations of participants of music events remain too few and disparate to evaluate the prevalence and the kind of NPS used at the Art-Scenyk Festival and the Garorock Festival in 2017.

The problem of many research concerning NPS is often the methodology and problems with the detection new substances. In this way, it is obvious that the present results may be questionable owing to several potential bias. The timeframe of NPS use and the impaired state duration were not documented in these NPS positive cases. In the same way, the delay between the time of drug use and the collection of oral fluid was unknown. There is a random sampling of OF links to roadside check decided by police officers. On an analytical point of view, beyond the lack of completeness and timeliness of the analytical method used, it is not possible to assess the limits of detection of the used methods mainly because the volume of analyzed OF is unknown. Indeed the initial volume of OF collected on the collection pad of the Drugwipe-5S® test is not precise (about 10 µL but can ranged from 5 to 50 µL) and a non-assessable part of it is used by the immuno-chromatographic system of the Drugwipe-5S® test. Consecutively, there is no control of the residual quantity of OF (remaining on the pad as DSS) that is really analysed. In addition, instability issues of NPS in OF (and mainly in DSS) before analysis cannot be excluded. As a result, many compounds could not be detected, and achieved results only show what was detected, but would not necessarily be representative of real NPS use (i.e. undervaluation of NPS use).

Nevertheless, it is legitimate to regard our results as a reflect of drug use at music festival (i) in the increased observed prevalence of NPS use (7.4%) compared with NPS prevalence in general population of drivers submitted to roadside screening test for DOA (3%), and (ii) in the kind of detected NPS (increase in the variety of used NPS and a large proportion of cyclohexanones: 7 out the 18 NPS detection). Finally, 5% of drivers initially roadside-tested negative for DOA were in fact driving after use NPS. These 7 drivers were again getting behind the wheel of their car after the roadside check.

Conclusion

The multitude of NPS, coupled with the continued emergence of novel NPS, challenge enforcement, testing and health agencies, as well as government and policy makers. This situation is complicated in driver populations as roadside immunological screening tests used for action against DUID are not adapted for NPS detection. In this way, these results confirm the reality of driving after NPS use in French drivers and suggest this phenomenon could concern even more people driving to or from a music festival.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Table and figure legends

Figure 1: Detected NPS together with number of identifications and connections (simultaneous presence) between NPS and DOA (black).

Table 1: Sample collection and NPS detection results in collected OF.

Figure 1

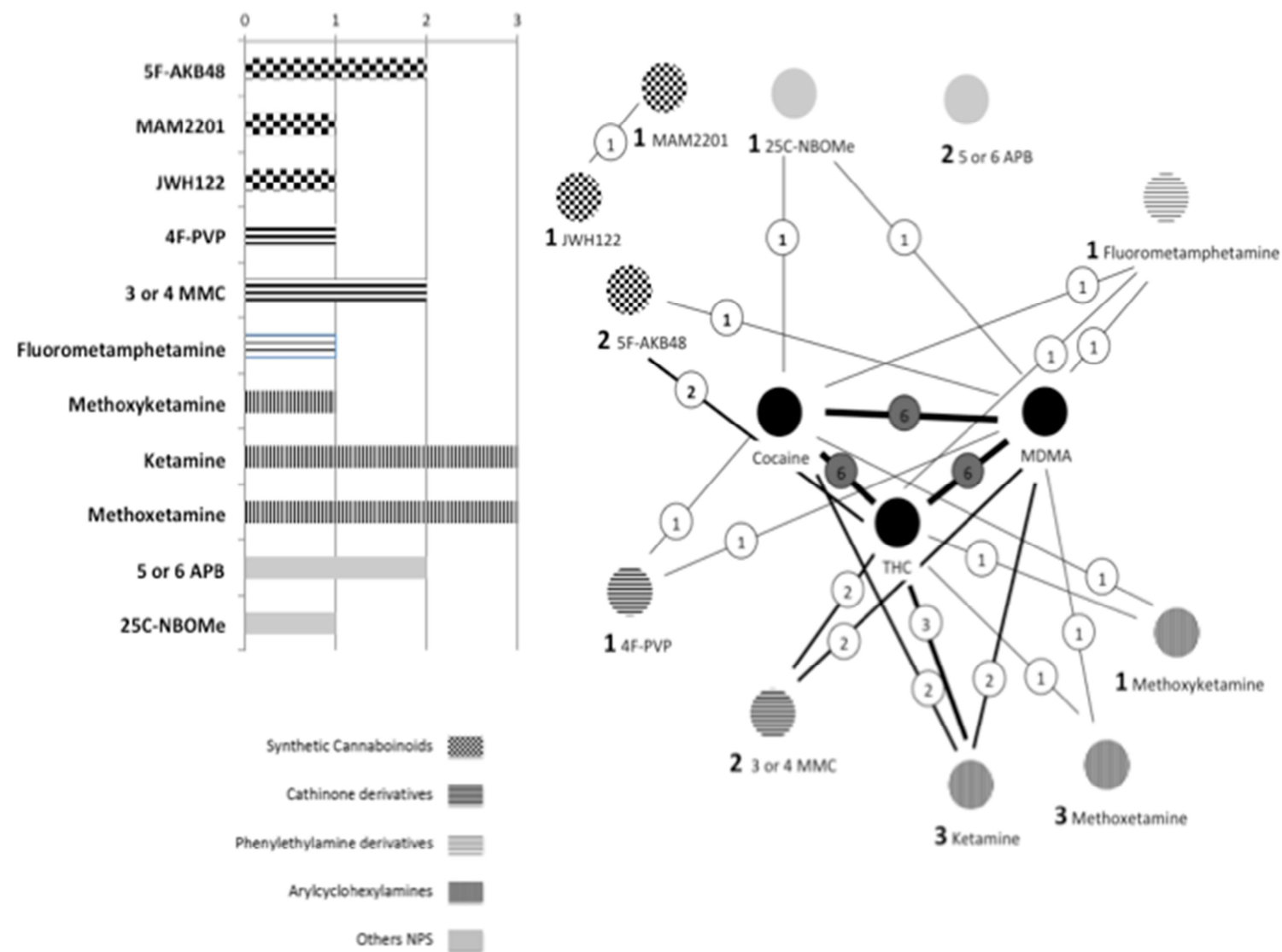
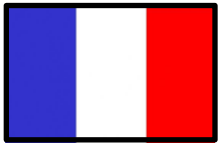


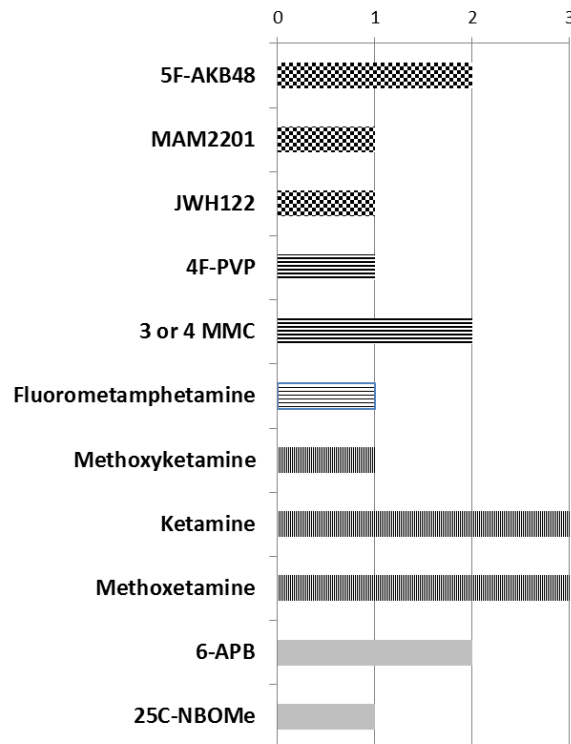
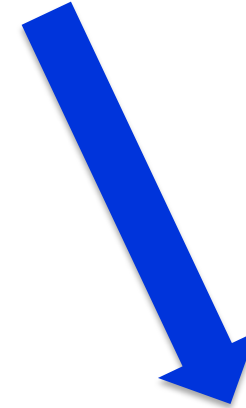
Table 1

	Collected DW	Collected DW entrant/leaver	Negative roadside tests for DOA ("Neg")	Total OF with NPS (%)	Identified NPS
Art Scenyck Festival	9	not available	2 (22%)	2 (22%)	3 or 4 MMC, 4F-PVP
Garorock Festival	220		131 (60%)	15 (6.8%)	
		68 entrants	41 (60%)	4 (5.9%)	Methoxyketamine, Methoxetamine
		152 leavers	90 (60%)	11 (7.2%)	6-APB, 25C-NBOMe, 3 or 4 MMC, Ketamine, Fluorometamphetamine, 5F-AKB48, MAM2201, JWH122
Total	229		133	17 (7.4%)	



2017

n: 229



NPS positivity rate of
7.4%
in oral fluid of drivers

