Underestimated impact of novel psychoactive substances: laboratory confirmation of recreational drug toxicity in Oslo, Norway

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Abstract

Context: Recreational drug toxicity is frequent. Availability of new psychoactive substances is steadily increasing. However, data with verified analyses from clinical settings are limited. To evaluate the impact of novel psychoactive substances (NPS) on recreational drug toxicity in Oslo, Norway, we analysed samples from a selection of patients.

Methods: All patients presenting with recreational drug toxicity at the Oslo Accident and Emergency Outpatient Clinic (OAEOC) and at the Oslo University Hospital (OUH) were registered from April through September 2014. Oral fluid samples were collected at the OAEOC. Blood samples were collected at the OUH. The samples were screened using ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS).

Results: 964 cases were included, 841 (87.2%) at the OAEOC and 123 (12.8%) at the OUH. A total of 55 oral fluid samples (OAEOC) and 103 blood samples (OUH) could be analysed. NPS were not clinically suspected in any of the screened cases. At the outpatient clinic, the most commonly found substances were clonazepam in 42/55 (76.4%) cases, amphetamines in 40/55 (72.7%) and heroin in 39/55 (70.9%). In seven (12.7%) cases NPS were detected: 4-methylamphetamine in three cases, dimethyltryptamine in two, methylone in one, and N,N-dimethyl-3,4-methylenedioxyamphetamine in one. Among the hospital patients, the most commonly found substances were clonazepam in 51/103 (49.5%) cases, amphetamines in 48/103 (46.6%), heroin in 31/103 (30.1%), and diazepam in 30/103 (29.1%). In five (4.9%) cases NPS were detected: JWH-210 in two cases, AM-2201 in two, and 5-EAPB in one.

Conclusion: NPS were clinically not suspected, though found in eight percent of cases. Still, the vast majority of patients treated for recreational drug toxicity in Oslo have taken classical drugs. Management of these patients should be based on their clinical condition. However, it is highly important to be alert to atypical presentations possibly resulting from unsuspected drugs.

Introduction

Recreational drug toxicity is a frequent cause of presentation to emergency departments (EDs) [1-3]. The availability of novel psychoactive substances (NPS) is steadily increasing, as is the number of different substances, with two hundred new substances reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) over the last two years [4, 5]. However, there are limited data on toxic exposures with verified analyses from clinical settings [6].

The diagnosis of toxic agents involved in recreational drug toxicity is based on clinical features and history. Toxicological analyses are time consuming, and results are not available to clinicians when treatment decisions have to be made. As the number of different NPS is ever increasing, even extensive laboratory screenings fail to keep up, and some substances will go undetected [7]. This problem applies to an even larger extent to regular toxicological screenings and point of care tests [7]. The limitations in toxicological laboratory testing, along with the known inaccuracies of the clinical diagnosis of toxic agents [8-10], make toxicovigilance concerning NPS difficult.

Objectives

To evaluate the impact of NPS on recreational drug toxicity in Oslo, Norway, we registered all patients treated for recreational drug toxicity at the Oslo Accident and Emergency Outpatient Clinic (OAEOC) and at Oslo University Hospital (OUH) during six months, and analysed oral fluid and whole blood samples obtained. In addition, we compared clinical diagnoses with the laboratory confirmation of toxic agents.

Materials and methods

The study was cross-sectional, including patients consecutively at two centres in Oslo, Norway, from 1 April to 30 September 2014.

Settings

The Norwegian emergency care system is two-tiered. Unless obviously critically ill, patients are initially assessed in primary care (emergency outpatient clinics, general practitioners, ambulance services), and only transferred to hospital when necessary. The OAEOC is an emergency outpatient clinic serving the entire city at all hours. The OAEOC has about 200

000 consultations a year. There are facilities for short time observation, but diagnostic tools and treatment options are limited. The OUH is one of four hospitals treating patients with acute poisoning in Oslo. About 4600 patients per year are treated in the ED at the OUH. In Oslo, the vast majority of patients with recreational drug toxicity are treated at the OAEOC, but the more severe cases, including most gamma-hydroxybutyrate (GHB) poisonings, are transferred to hospital or brought directly to hospital by the ambulance service.

Inclusion

All patients presenting with recreational drug toxicity to any of the two participating centres were included. Recreational drug toxicity was defined as symptoms and/or signs of acute toxicity with any psychoactive compound taken for recreational purposes rather than for medical or work purposes or as part of deliberate self-harm, including classic recreational drugs, new psychoactive substances, prescription pharmaceuticals, plants, fungi, herbal medicines, and industrial and/or domestic products. Patients with alcohol as the sole toxic agent were not included, but patients with recreational drug toxicity combined with alcohol were. Patients with additional diagnoses were included if the recreational drug toxicity in itself warranted treatment. Patients transferred from the OAEOC to the OUH were registered as OUH patients only.

Data collection

Cases were identified retrospectively from electronic patient registration lists. Data were collected as part of the European Drug Emergencies Network (Euro-DEN) and extracted from electronic patient records using the Euro-DEN minimum dataset, as described elsewhere [11]. Age, gender, toxic agents, whether the patient was brought by ambulance, length of stay, disposition (medically discharged, self discharge, admitted intensive care unit, admitted psychiatric ward, admitted other hospital ward, death), observations on presentation (lactate (hospital ED only), temperature, glucose, level of consciousness (Glasgow Coma Scale score), heart rate, blood pressure, respiratory rate), clinical features (vomiting, dyspnoea, hyperthermia, headache, anxiety, hallucinations, agitation/aggression, psychosis, seizures, cerebellar features, palpitations, chest pain, hypertension, hypotension, arrhythmias), QRS and QTc values from electrocardiography, peak creatine kinase, peak creatinine, and treatment were registered.

Diagnosis of toxic agents was based on the assessment of the clinician treating the patient as stated in the electronic patient records. The clinicians' assessments were based on all information available to them; patient self-report and history, information from whoever brought the patient to the emergency department, clinical examination and regular laboratory tests. Clinical diagnoses of amphetamine or methamphetamine were co-categorised as amphetamines.

Toxicological sampling and analyses

Collecting oral fluid samples at the outpatient clinic required obtaining informed consent. Drowsy, agitated or disoriented patients were not approached until coming round and considered able to consent. Consenting patients were included prospectively. Two nurses were assigned for this task, and samples were only collected at the OAOEC when they were present. Samples were collected on 40 shifts, including 22 weekend shifts. In the hospital ED whole blood samples were routinely obtained on presentation from all patients treated for recreational drug toxicity. However, some patients refused giving blood samples or left the ED before samples were taken.

Whole blood was screened at the Department of Forensic Sciences using ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) for classical drugs-of-abuse [12], modified to include designer benzodiazepines. Synthetic cannabinoid receptor agonists [13] and a wide range of other new psychoactive substances (cathinones, tryptamines, phenethylamines, designer opioids) were screened for using the same technique. The oral fluid drug screening covered the same compounds using published methods of UHPLC-MS/MS for drugs of abuse and synthetic cannabinoids [14-16], and a similar method for NPS as for whole blood. Alcohol was screened for with an automated enzymatic method using alcohol dehydrogenase [17]. Cut off values are provided in Supplementary Table 1.

GHB was analysed in oral fluid in the same manner as previously published for blood [18]. Important analytical parameters as linearity and accuracy were specifically tested for oral fluid. Blood samples were not screened for GHB, as the primary focus of the analysis at the Department of Forensic Sciences was NPS, although other common recreational drugs that could be analysed with the same methods as the NPS were included. Furthermore, in many

cases only limited sample material was available. The known short detection time of GHB in whole blood made the detection in blood less likely, and urine was not available for analysis. However a small number of urine specimens were tested for GHB, when clinically suspected by the physician in the hospital ED, by a specific gas cromatography mass spectrometry (GCMS) method. We searched the records of the included patients for the test results.

In the oral fluid and blood samples, amphetamine and methamphetamine were co-categorised as amphetamines. In the oral fluid samples, 6-monoacetylmorphine (6-MAM) and morphine were categorised as heroin. In the blood samples, morphine-6-glucuronide (M6G), morphine-3-glucuronide (M3G) and morphine were categorised as heroin. Otherwise, specific metabolites were categorised as the parent compound, also when the latter was absent.

If a substance was detected in a sample in a case where the same substance had been given as treatment, the substance was not counted as a recreational drug. This pertained to ten cases of diazepam, nine from the hospital ED and one from the outpatient clinic, and one case of ketamine from the hospital ED.

Ethics

The study was performed in accordance with the Helsinki declaration. It was approved by the Oslo University Hospital Information Security and Privacy Office and by the Regional Committee South East for Medical and Health Research Ethics (REK sør-øst D 2014/116).

Statistics

The Excel files were converted to SPSS, and analyses were done in IBM SPSS version 23 (IBM Corp, Chicago, Illinois, USA.) and in an online calculator from Epitools [19]. Pearson's chi-square test or Fisher's exact test (for expected cell counts of five or less) were used to compare frequencies. Mann-Whitney U-test was used in comparisons of age, length of stay and number of drugs suspected taken between screened and non-screened cases. Wilcoxon signed ranks test was used when comparing number of drugs suspected and substances found on screening.

Results

In total, 964 cases were included, 841 (87.2 %) at the outpatient clinic and 123 (12.8 %) in the hospital ED (Figure 1), 754 (78.2 %) were males. Median age was 33 years (interquartile range (IQR) 26-44). The most frequent clinically suspected agents at the outpatient clinic were heroin in 407/841 (48.4 %) cases, amphetamines in 174/841 (20.7 %) and clonazepam in 154/841 (18.3 %) (Table 1). The most frequent clinically suspected agents among the hospital patients were GHB in 51/123 (41.5 %) cases, heroin in 34/123 (27.6 %) and amphetamines in 33/123 (26.8 %) (Table 2). Co-ingestion of alcohol was reported in 226/841 (26.9 %) cases at the outpatient clinic and in 46/123 (37.4 %) cases in the hospital ED. NPS were not clinically suspected in any of the screened cases, though suspected in seven of the 786 (0.9 %) non-screened cases at the outpatient clinic.

A total of 105 blood samples were collected in the hospital ED. Two samples did not contain enough material for analysis, leaving 103 analysed samples (among 123 cases; 83.7 %). Median time from presentation to sampling was 32 min (IQR 13 min – 73 min). Time from taking drugs to presentation at the hospital ED was 1-5 hours in 32/103 (31.1 %) cases, 5 hours or more in 27/103 (26.2 %) cases, and unknown in 44/103 (42.7 %) cases. At the outpatient clinic, 55 oral fluid samples were collected and analysed (among 841 cases; 6.5 %). Timing of the samples was not recorded at the outpatient clinic. However, as samples could not be taken until the patient was able to consent, most samples were taken during the last hour before discharge. Median length of stay among sampled patients at the outpatient clinic was 5 h 54 min (IQR 4 h 12 min – 7 h 2 min). Time from taking drugs to presentation was less than one hour in 1/55 (1.8 %) cases, 1-5 hours in 8/55 (14.5 %) cases, 5 hours or more in 2/55 (3.6 %) cases, and unknown in 44/55 (80.0 %) cases.

At the outpatient clinic, the most commonly found substances were clonazepam in 42/55 (76.4 %) cases, amphetamines in 40/55 (72.7 %) heroin in 39/55 (70.9 %) (Table 1). The median number of substances per sample was five (range 1-9), not counting metabolites. There were 30/55 (54.5 %) cases of combined amphetamines and heroin, 29 of them also with benzodiazepines. NPS were detected in 7/55 (12.7 %) cases (Table 3).

Among the hospital patients, the most commonly found substances were clonazepam in 51/103 (49.5 %) cases, amphetamines in 48/103 (46.6 %) and heroin in 31/103 (30.1 %) (Table 2). The median number of substances per sample was two (range 0-8). There were

19/103 (18.4 %) cases of combined amphetamines and heroin, 18 of them also with benzodiazepines. NPS were detected in 5/103 (4.9 %) cases (Table 3).

The majority of clinically suspected heroin, amphetamines and clonazepam cases were confirmed (Tables 1 & 2), as were most cannabis cases at the outpatient clinic. Among the hospital patients, cannabis was suspected in 12 cases and not found in eight (66.7 %) of them. There were 14 cases with no substances found, all of them among the hospital patients. In general, a larger number of substances was found in the samples than was clinically suspected ($p < 0.001$ both at the outpatient clinic and in the hospital ED).

GHB was a suspected agent in five cases at the outpatient clinic, among whom four were confirmed. In the hospital ED, where GHB was not screened for in the blood samples, it was a suspected agent in 45 screened cases. Among the 24 cases where GHB was the only suspected agent, a combination of stimulants and depressants was found in 11 cases, depressants in six, amphetamines in one, and in six cases no substances were found. In the hospital ED, urine specimens were tested for GHB in five cases when clinically suspected. Two tests were positive. In both cases several other agents were found in the blood sample screening.

Discussion

Though not clinically suspected in any of the screened patients, NPS were found in eight percent of cases. Laboratory analyses confirmed the dominance of the classical recreational drugs heroin, amphetamines and benzodiazepines among patients treated for acute recreational drug toxicity in Oslo. More substances were found on screening than clinically suspected. Heroin, amphetamines and clonazepam were mostly confirmed when suspected, as was cannabis at the outpatient clinic.

The presence of NPS was clinically underestimated. Among screened cases NPS were found in eight percent, though suspected in none. Furthermore, NPS were clinically suspected in less than one percent of non-screened cases. The underestimation may be due to clinicians not being alert to NPS and hence not asking patients specifically, or it may be related to the general underestimation of the number of agents taken demonstrated both in this and previous

studies [9, 10]. It is also possible that the patients themselves do not know which substances their drug preparations contain.

We found seven different NPS: 4-methylamphetamine (4-MA), a phenethylamine derivative involved in several deaths in 2011-12 in Belgium, the Netherlands, Denmark and the UK [20, 21]. 4-MA is often sold as amphetamine, or in preparations containing both 4-MA and amphetamine [21]. In two of our three cases both substances were found. N,N-dimethyl-3,4-methylenedioxyamphetamine (N,N-dimethyl-MDA), another phenethylamine derivative [22]. 5-(2-ethylaminopropyl)benzofuran (5-EAPB), a benzofuran phenethylamine derivative, probably with effects similar to 3,4-methylenedioxymethamphetamine (MDMA) [23]. Methylone, a synthetic cathinone also sharing many properties with MDMA [24]. JWH-210 and AM-2201, both synthetic cannabinoid receptor agonists, the latter previously reported in Norway in suspected impaired drivers [25-27]. Finally, dimethyltryptamine (DMT), a hallucinogenic indole alkaloid and one of the constituents of ayahuasca, a botanical preparation used for ritual and therapeutic purposes in the Amazon [28, 29].

In most NPS cases we also found a variety of both stimulants and depressants. Thus, we are unable to discern what clinical effects were due to the NPS. We have not found any clinical description of toxicity from N,N-dimethyl-MDA in the literature. Our patient was agitated and tachycardic, and MDMA was the only other substance found in the oral fluid sample. It is possible that N,N-dimethyl-MDA was an impurity in the the MDMA taken, and both substances probably contributed to the clinical picture. The hyperthermia seen in one of the JWH-210 cases, where heroin also was found, was probably caused by a concurrent groin abscess.

In general, classical recreational drugs dominated. This is consistent both with previous local studies [2, 3, 30] and a European multi-centre study [1] based on self-report of drugs taken. In the latter study the frequency of reported NPS varied between centres and was concentrated in London, York, Dublin, Munich and Gdansk, where the proportions of reported NPS among recreational drug toxicity presentations ranged from 11.4 % to 30.6 % [1, 31]. Both the OAEOC and the OUH contributed data to this multi-centre study, including the cases in the present study, and NPS proportions were 0.6 % and 0.5 % at the two Oslo centres respectively [31]. In two Swiss case series from 2013-15, blood samples from a total of 178 patients treated for acute recreational drug toxicity screened with LC-MS/MS rendered only classical

recreational drugs except one case of pentylone [32, 33]. Synthetic cannabinoid receptor agonists were not screened for in these case series.

We consistently found more and different drugs in the laboratory analyses than were clinically suspected. This may be due to widespread use of multiple drugs simultaneously or sequentially over short periods of time. Another explanation is that drugs can be traceable in blood and oral fluid samples for some time after the clinical effects have worn off. The number of substances detected depends on substances taken, the dose, the time between intake and sample collection, elimination time of the drug and the analytical cut-off applied for each medium. Co-findings from previous intakes, as well as undetected substances due to low concentrations in the analysed medium, are therefore not surprising. In our analysis of the blood samples, we used a lower cut-off level than usual. This resulted in nine samples with trace findings being counted as positive. All the agents thus confirmed were clinically suspected.

The combination of heroin, amphetamines and benzodiapines was prominent, especially at the outpatient clinic. In our experience, these patients have poor health and mainly inject their drugs. The combination of stimulants and depressants may have unpredictable effects, contributing to the dangers of polydrug use. Ninety percent of drug-induced deaths in Oslo in 2006-8 were polydrug poisonings [34].

Ketobemidone was found in two cases at the hospital ED. Though not much in use elsewhere, it is a prescription drug in the Nordic countries and has been reported in opioid overdoses [35, 36].

It may seem that the clinical diagnosis of toxic agents is not very sensitive as many substances found in the laboratory analyses were not suspected. On the other hand, in current best practice in clinical toxicology, management of patients with recreational drug toxicity is mainly based on assessment of the patients' clinical condition and recognising toxidromes rather than the treatment of specific toxic effects of specific drugs. Though the patients have taken several drugs, only one or two may be the main agents behind the condition leading to presentation to the emergency services. Thus, the clinical diagnosis of toxic agents will not be exhaustive, but based on the clinical picture needing observation or treatment. In keeping with

this, we found that clinically suspected drugs often were confirmed in the laboratory analyses, with exceptions for cannabis and cocaine among the hospital patients.

Limitations

Despite the extensive library used for screening in our study, our patients may have taken drugs we did not screen for. In some samples, limited amount of blood made screening of the full repertoire difficult. Hence, nine blood samples were not screened for synthetic cannabinoid receptor agonists. Screening for cannabis was not performed by a dedicated method, but together with many other substances. This might not have given the necessary sensitivity to confirm cannabis intake when the time between intake and blood sampling was long.

We did not screen for GHB among the hospital patients. GHB was suspected in a large proportion of the hospital cases, and may cause a variety of symptoms, mimicking both stimulant and depressant toxidromes [37]. However, the impact of GHB in our case series remains uncertain. While four of the five suspected outpatient cases were confirmed, only two of the five urine specimens from suspected hospital cases tested positive on GHB.

While samples were obtained from the vast majority of eligible hospital ED patients, the screening at the outpatient clinic was done on a convenience sample. However, apart from screened patients less frequently presenting with psychosis and more frequently being medically discharged, there were no significant differences in age, gender, observations on presentation, clinical features, treatment, disposition or the toxic agents suspected between the screened and non-screened cases at the outpatient clinic. Psychotic patients were considered unable to give informed consent. Hence, they were not screened. This is probably the main reason why no screened patients were admitted to a psychiatric ward from the outpatient clinic.

Some of the benzodiazepines, prescription opioids and quetiapine found in the samples may have been the patients' prescribed medication. However, in most cases these substances appeared in combinations with obvious recreational drugs, and were probably taken for recreational purposes.

We co-categorised amphetamine and methamphetamine as amphetamines, both when reporting the clinical diagnoses and the laboratory results. This was done as discerning between the two is difficult. Methamphetamine is metabolised to amphetamine, and the two substances usually occured together in the samples. Furthermore, users in Oslo do not distinguish between them. During the last decade methamphetamine has been as prevalent as amphetamine in Norway [38].

When reporting the laboratory results, we co-categorised 6-MAM, M6G, M3G and morphine as heroin. Codeine was reported separately. Codeine occurs naturally in the opium poppy, and may be found in illicit heroin [39]. As both heroin and codeine are metabolised to morphine, it is difficult to differentiate between codeine from medical preparations and from heroin. Codeine was rarely a suspected toxic agent compared to heroin in our study. Thus, it is likely that most codeine cases stemmed from illicit heroin.

Conclusion

NPS were clinically rarely suspected, though found in eight percent of screened cases. Still, the vast majority of the patients treated for recreational drug toxicity in Oslo have taken classical drugs. We found a dominance of amphetamines among the stimulants, and of heroin and benzodiazepines (especially clonazepam) among the sedatives.

Suspected toxic agents were often confirmed, but we consistently found more toxic agents in the samples than was clinically diagnosed. While the management of patients presenting with recreational drug toxicity should be based on their clinical condition, it is highly important to be alert to atypical presentations possibly resulting from unsuspected and/or multiple drugs.

Conflict of interest

We have no potential conflict of interest to report.

References

1. Dines AM, Wood DM, Yates C, et al. Acute recreational drug and new psychoactive substance toxicity in Europe: 12 months data collection from the European Drug Emergencies Network (Euro-DEN). Clin Toxicol. 2015;53:893-900.
2. Lund C, Drottning P, Stiksrud B, et al. A one-year observational study of all hospitalized acute poisonings in Oslo: complications, treatment and sequelae. Scand J Trauma Resusc Emerg Med. 2012;20:49.
3. Vallersnes OM, Jacobsen D, Ekeberg O, et al. Patients presenting with acute poisoning to an outpatient emergency clinic: a one-year observational study in Oslo, Norway. BMC Emerg Med. 2015;15:18.
4. European monitoring centre for drugs and drug addiction. European Drug Report 2015. Trends and developments. Lisbon, Portugal: EMCDDA, 2015.
5. European monitoring centre for drugs and drug addiction. European Drug Report 2016. Trends and developments. Lisbon, Portugal: EMCDDA, 2016.
6. Heyerdahl F, Hovda KE, Giraudon I, et al. Current European data collection on emergency department presentations with acute recreational drug toxicity: gaps and national variations. Clin Toxicol. 2014;52:1005-12.
7. Peters FT, Meyer MF. Analytical techniques for the detection of novel psychoactive substances and their metabolites. In: Dargan PI, Wood DM, editors. Novel psychoactive substances: classification, pharmacology and toxicology. London, UK: Academic Press; 2013. p. 131-57.
8. Heyerdahl F, Hovda KE, Bjornaas MA, et al. Clinical assessment compared to laboratory screening in acutely poisoned patients. Hum Exp Toxicol. 2008;27:73-9.
9. Mordal J, Holm B, Morland J, et al. Recent substance intake among patients admitted to acute psychiatric wards: physician's assessment and on-site urine testing compared with comprehensive laboratory analyses. J Clin Psychopharmacol. 2010;30:455-9.
10. Bjornaas MA, Hovda KE, Mikalsen H, et al. Clinical vs. laboratory identification of drugs of abuse in patients admitted for acute poisoning. Clin Toxicol. 2006;44:127-34.
11. Wood DM, Heyerdahl F, Yates CB, et al. The European Drug Emergencies Network (Euro-DEN). Clin Toxicol. 2014;52:239-41.

12. Oiestad EL, Johansen U, Oiestad AM, et al. Drug screening of whole blood by ultra-performance liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 2011;35:280-93.

13. Karinen R, Johnsen L, Andresen W, et al. Stability study of fifteen synthetic cannabinoids of aminoalkylindole type in whole blood, stored in Vacutainer® evacuated glass tubes. J Forensic Toxicol Pharmacol. 2013;2:1.

14. Oiestad EL, Johansen U, Christophersen AS, et al. Screening of synthetic cannabinoids in preserved oral fluid by UPLC-MS/MS. Bioanalysis. 2013;5:2257-68.

15. Lund HM, Oiestad EL, Gjerde H, et al. Drugs of abuse in oral fluid collected by two different sample kits: stability testing and validation using ultra performance tandem mass spectrometry analysis. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879:3367-77.

16. Oiestad EL, Johansen U, Christophersen AS. Drug screening of preserved oral fluid by liquid chromatography-tandem mass spectrometry. Clin Chem. 2007;53:300-9.

17. Kristoffersen L, Smith-Kielland A. An automated alcohol dehydrogenase method for ethanol quantification in urine and whole blood. J Anal Toxicol. 2005;29:387-9.

18. Dahl SR, Olsen KM, Strand DH. Determination of gamma-hydroxybutyrate (GHB), beta-hydroxybutyrate (BHB), pregabalin, 1,4-butane-diol (1,4BD) and gamma-butyrolactone (GBL) in whole blood and urine samples by UPLC-MSMS. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;885-886:37-42.

19. Sergeant ESG. Epitools epidemiological calculators [Internet]. Australia: AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease; 2016 [Cited 8 January 2016]. Available from: http://epitools.ausvet.com.au/.

20. Blanckaert P, van Amsterdam J, Brunt T, et al. 4-Methyl-amphetamine: a health threat for recreational amphetamine users. J Psychopharmacol. 2013;27:817-22.

21. European Monitoring Centre for Drugs and Drug Addiction. 4-Methylamphetamine (4-MA): report on the risk assessment of 4-methylamphetamine in the framework of the Council Decision on new psychoactive substances. Luxembourg: EMCDDA, 2014.

22. Cody JT. Detection of D,L-amphetamine, D,L-methamphetamine, and illicit amphetamine analogs using diagnostic products corporation's amphetamine and methamphetamine radioimmunoassay. J Anal Toxicol. 1990;14:321-4.

23. Rickli A, Kopf S, Hoener MC, et al. Pharmacological profile of novel psychoactive benzofurans. Br J Pharmacol. 2015;172:3412-25.

24. Karila L, Billieux J, Benyamina A, et al. The effects and risks associated to mephedrone and methylone in humans: A review of the preliminary evidences. Brain Res Bull. 2016;126:61-7.

25. Obafemi AI, Kleinschmidt K, Goto C, et al. Cluster of acute toxicity from ingestion of synthetic cannabinoid-laced brownies. J Med Toxicol. 2015;11:426-9.

26. Tuv SS, Krabseth H, Karinen R, et al. Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period. Accid Anal Prev. 2014;62:26-31.

27. Hermanns-Clausen M, Kithinji J, Spehl M, et al. Adverse effects after the use of JWH-210: a case series from the EU Spice II plus project. Drug Test Anal. 2016;8:1030-8.

28. Dos Santos RG, Balthazar FM, Bouso JC, et al. The current state of research on ayahuasca: a systematic review of human studies assessing psychiatric symptoms, neuropsychological functioning, and neuroimaging. J Psychopharmacol. 2016;30:1230-47.

29. Carbonaro TM, Gatch MB. Neuropharmacology of N,N-dimethyltryptamine. Brain Res Bull. 2016;126:74-88.

30. Lund C, Vallersnes OM, Jacobsen D, et al. Outpatient treatment of acute poisonings in Oslo: poisoning pattern, factors associated with hospitalization, and mortality. Scand J Trauma Resusc Emerg Med. 2012;20:1.

31. Euro-DEN Research Group. Final Report of the European Drug Emergencies Network (Euro-DEN). London, UK: 2015.

32. Liakoni E, Dolder PC, Rentsch KM, et al. Presentations due to acute toxicity of psychoactive substances in an urban emergency department in Switzerland: a case series. BMC Pharmacol Toxicol. 2016;17:25.

33. Liakoni E, Dolder PC, Rentsch K, et al. Acute health problems due to recreational drug use in patients presenting to an urban emergency department in Switzerland. Swiss Med Wkly. 2015;145:w14166.

34. Gjersing L, Jonassen KV, Biong S, et al. Diversity in causes and characteristics of drug-induced deaths in an urban setting. Scand J Public Health. 2013;41:119-25.

35. Steentoft A, Teige B, Holmgren P, et al. Fatal poisonings in young drug addicts in the Nordic countries: a comparison between 1984-1985 and 1991. Forensic Sci Int. 1996;78:29-37.

36. Jonsson A, Holmgren P, Ahlner J. Fatal intoxications in a Swedish forensic autopsy material during 1992-2002. Forensic Sci Int. 2004;143:53-9.

37. Schep LJ, Knudsen K, Slaughter RJ, et al. The clinical toxicology of gamma-hydroxybutyrate, gamma-butyrolactone and 1,4-butanediol. Clin Toxicol. 2012;50:458-70.

38. Bramness JG, Reid MJ, Solvik KF, et al. Recent trends in the availability and use of amphetamine and methamphetamine in Norway. Forensic Sci Intl. 2015;246:92-7.

39. Paterson S, Cordero R. Comparison of the various opiate alkaloid contaminants and their metabolites found in illicit heroin with 6-monoacetyl morphine as indicators of heroin ingestion. J Anal Toxicol. 2006;30:267-73.

Table 1. Cases at the outpatient clinic

Toxic agents	Non-screened cases	Screened cases			
	Suspected n (%)	Suspected n (%)	Confirmed when suspected n (%)	Confirmed when not suspected n (%)	Confirmed total n (%)
Clonazepam	141 (17.9)	13 (23.6)	13 (23.6)	29 (52.7)	42 (76.4)
Amphetamines	166 (21.1)	8 (14.5)	7 (12.7)	33 (60.0)	40 (72.7)
Heroin	376 (47.8)	31 (56.4)	30 (54.5)	9 (16.4)	39 (70.9)
Codeine	4 (0.5)	2 (3.6)	2 (3.6)	30 (54.5)	32 (58.2)
Cannabis	93 (11.8)	12 (21.8)	10 (18.2)	14 (25.5)	24 (43.6)
Alprazolam	33 (4.2)	3 (5.5)	1 (1.8)[c]	18 (32.7)	19 (34.5)
Diazepam	42 (5.3)	3 (5.5)	2 (3.6)[d]	14 (25.5)	16 (29.1)
Methadone	22 (2.8)	2 (3.6)	2 (3.6)	13 (23.6)	15 (27.3)
Cocaine	43 (5.5)	3 (5.5)	2 (3.6)	6 (10.9)	8 (14.5)
Oxazepam	20 (2.5)	1 (1.8)	-[e]	8 (14.5)	8 (14.5)
NPS	7 (0.9)[b]	-	-	7 (12.7)[h]	7 (12.7)[h]
Nitrazepam	5 (0.6)	2 (3.6)	2 (3.6)	4 (7.3)	6 (10.9)
GHB	68 (8.7)	5 (9.1)	4 (7.3)	-	4 (7.3)
MDMA	20 (2.5)	1 (1.8)	1 (1.8)	2 (3.6)	3 (5.5)
Buprenorphine	15 (1.9)	4 (7.3)	-	3 (5.5)	3 (5.5)
Z-drugs	7 (0.9)	-	-	2 (3.6)	2 (3.6)
Oxycodone	3 (0.4)	-	-	1 (1.8)	1 (1.8)
Morphine	14 (1.8)	-	-	-	-
LSD	8 (1.0)	-	-	-	-
Flunitrazepam	6 (0.8)	-	-	-	-
Psilocybe mushrooms	4 (0.5)	-	-	-	-
Methylphenidate	3 (0.4)	-	-	-	-
Pregabalin	3 (0.4)	-	-	-	-
Tramadol	2 (0.3)	-	-	-	-
Quetiapine	2 (0.3)	-	-	-	-
Ketamine	1 (0.1)	1 (1.8)	-	-	-
PCP	1 0.1)	-	-	-	-
MDA	-	1 (1.8)	-	-	-
Unspecified benzodiazepine	31 (3.9)	2 (3.6)	2 (3.6)[f]	-	-
Unspecified opioid	23 (2.9)	-	-	-	-
Unspecified stimulant	4 (0.5)	-	-	-	-
Unknown	20 (2.5)	3 (5.5)	2 (3.6)[g]	-	-
Alcohol co-ingested	209 (26.6)	17 (30.9)			
Number of agents[a]	1 (1-2) (1-6)	1 (1-2) (1-4)			5 (3-7) (1-9)
Total	786 (100)	55 (100)	55 (100)	55 (100)	55 (100)

[a]Median (interquartile range) (absolute range), excluding alcohol.
[b]DMT suspected in two cases, 5-MeO-MiPT, 2C-B, 2C-E, SCRA and unspecified NPS in one case each.
[c]In the two unconfirmed alprazolam cases, clonazepam was found in both, and oxazepam in one.
[d]In one case of unconfirmed diazepam, clonazepam was found.
[e]In the unconfirmed oxazepam case, diazepam and alprazolam was found.
[f]Clonazepam in both, diazepam in one.
[g]Amphetamine and methadone in one, amphetamine, heroin and zopiclone in the other.
[h]4-MA in three cases, DMT in two, methylone and N,N-dimethyl-MDA in one each.

2C-B: 2,5-dimethoxy-4-bromophenethylamine, 2C-E: 2,5-dimethoxy-4-ethylphenethylamine, 4-MA: 4-methylamphetamine, 5-MeO-MiPT: 5-methoxy-N-methyl-N-isopropyltryptamine, DMT: dimethyltryptamine, GHB: gamma-hydroxybutyrate, LSD: lysergic acid diethylamide, MDA: 3,4-methylenedioxyamphetamine, MDMA: 3,4-methylenedioxymethamphetamine, N,N-dimethyl-MDA: N,N-dimethyl-3,4-methylenedioxyamphetamine, NPS: novel psychoactive substances, PCP: phencyclidine, SCRA: synthetic cannabinoid receptor agonists.

Table 2. Cases among hospital patients

Toxic agents	Non-screened cases	Screened cases			
	Suspected n (%)	Suspected n (%)	Confirmed when suspected n (%)	Confirmed when not suspected n (%)	Confirmed total n (%)
Clonazepam	-	8 (7.8)	7 (6.8)[b]	44 (42.7)	51 (49.5)
Amphetamines	2 (10.0)	31 (30.1)	26 (25.2)	22 (21.4)	48 (46.6)
Heroin	8 (40.0)	26 (25.2)	19 (18.4)	12 (11.7)[g]	31 (30.1)
Diazepam	-	-	-	30 (29.1)	30 (29.1)
Alprazolam	2 (10.0)	-	-	21 (20.4)	21 (20.4)
Codeine	-	1 (1.0)	-	16 (15.5)	16 (15.5)
Cannabis	1 (5.0)	12 (11.7)	4 (3.9)	11 (10.7)	15 (14.6)
Cocaine	4 (20.0)	11 (10.7)	7 (6.8)	4 (3.9)	11 (10.7)
Methadone	1 (5.0)	-	-	11 (10.7)	11 (10.7)
MDMA	-	3 (2.9)	2 (1.9)	3 (2.9)	5 (4.9)
Buprenorphine	-	-	-	5 (4.9)	5 (4.9)
NPS	-	-	-	5 (4.9)[h]	5 (4.9)[h]
Oxazepam	-	-	-	5 (4.9)	5 (4.9)
Fentanyl	-	-	-	4 (3.9)	4 (3.9)
Quetiapine	-	-	-	4 (3.9)	4 (3.9)
Ketamine	-	-	-	3 (2.9)	3 (2.9)
Nitrazepam	-	-	-	3 (2.9)	3 (2.9)
Z-drugs	-	-	-	3 (2.9)	3 (2.9)
Ketobemidone	-	-	-	2 (1.9)	2 (1.9)
Noscapine	-	-	-	2 (1.9)	2 (1.9)
Tramadol	-	-	-	1 (1.0)	1 (1.0)
GHB	6 (30.0)	45 (43.7)	-[c]	-[c]	-[c]
LSD	-	1 (1.0)	-	-	-
Sodium nitrite	-	1 (1.0)	-	-	-
Psilocybe mushrooms	1 (5.0)	-	-	-	-
Unspecified benzodiazepine	-	4 (3.9)	2 (1.9)[d]	-	-
Unspecified opioid	-	4 (3.9)	2 (1.9)[e]	-	-
Unknown	4 (20.0)	6 (5.8)	4 (3.9)[f]	-	-
Alcohol co-ingested	6 (30.0)	40 (38.8)			
Number of agents[a]	1 (1-2) (1-3)	1 (1-2) (1-5)			2 (1-4) (0-8)
Total	20 (100)	103 (100)	103 (100)	103 (100)	103 (100)

[a]Median (interquartile range) (absolute range), excluding alcohol.
[b]In one case of unconfirmed clonazepam, alprazolam was found.
[c]Not screened for in blood samples from the hospital patients.
[d]Clonazepam in both, diazepam and oxazepam in one, alprazolam in the other.
[e]Heroin and methadone in both, codeine in one.
[f]Heroin and fentanyl in one case, methadone in one, ketamine in one and cannabis in one.
[g]In three of the unconfirmed heroin cases, methadone was found in two and ketobemidone in one.
[h]JWH-210 and AM-2201 in two cases each, 5-EAPB in one.

5-EAPB: 5-(2-ethylaminopropyl)benzofuran, AM-2201: (1-(5-fluoropentyl)-3-(1-naphthoyl)indole), GHB: gamma-hydroxybutyrate, JWH-210: 4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone, LSD: lysergic acid diethylamide, MDMA: 3,4-methylenedioxymethamphetamine, NPS: novel psychoactive substances.

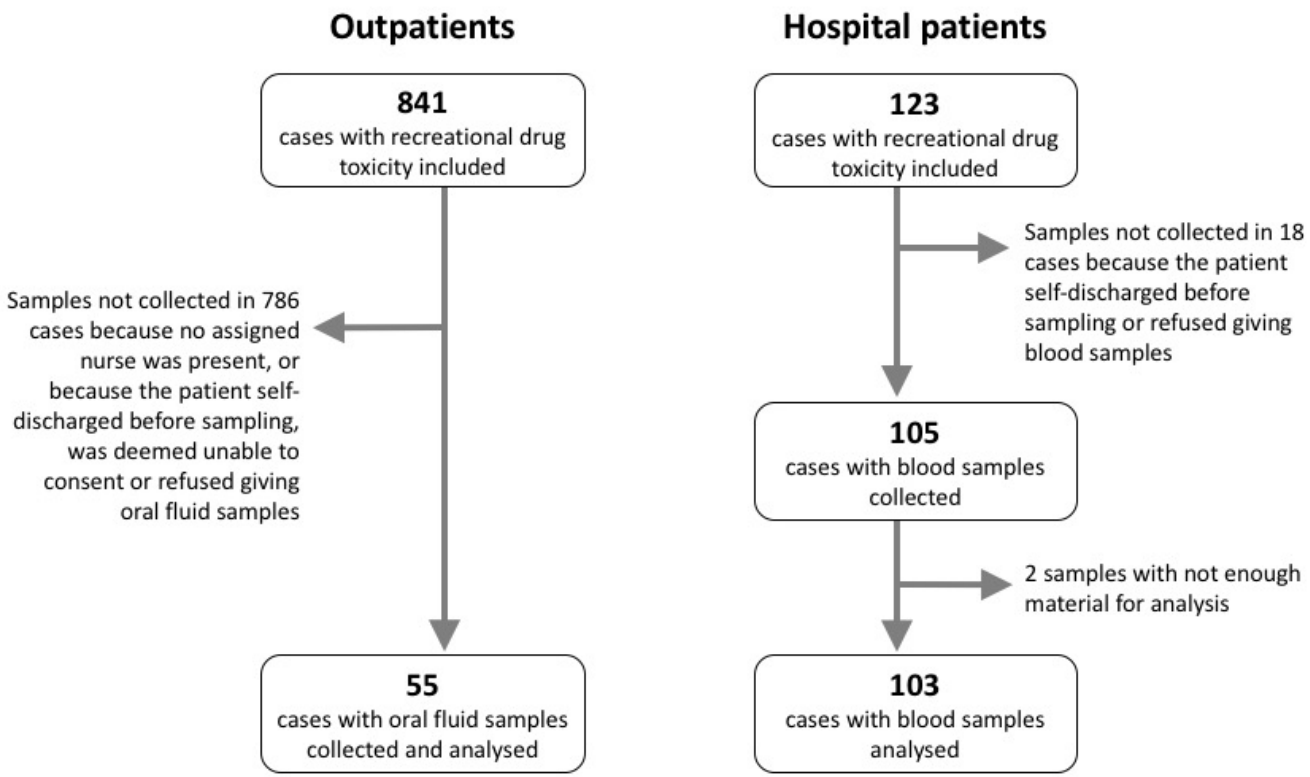
Table 3. Novel psychoactive substances found

Gender Age	NPS	Additional confirmed agents	Clinically suspected agents	Vital signs at presentation	Clinical features	Treatment	Length of stay (Hrs:min)	Disposition
M 41	4-MA	Amphetamines, THC, heroin, diazepam, clonazepam, methadone	Heroin	RR 11, HR 65, BP 125/87, temp 35.4°, GCS 13			6:57	Medically discharged
M 40	4-MA	Cocaine, heroin, oxazepam, alprazolam, clonazepam, nitrazepam	Heroin	RR 16, HR 116, BP 130/80, temp 37.0°, GCS 15			8:32	Medically discharged
F 36	4-MA	Amphetamines, heroin, clonazepam, nitrazepam	Heroin, amphetamines, clonazepam, nitrazepam	RR 18, HR 60, BP 130/70, temp 36.6°, GCS 15	Vomiting	Naloxone	5:54	Medically discharged
M 32	DMT	Cocaine, amphetamines, heroin, MDMA, oxazepam, clonazepam	Heroin	RR 12, HR 100, BP 136/79, temp 36.0°, GCS 15		Naloxone	5:46	Medically discharged
M 26	DMT	GHB, ethanol, MDMA, cocaine, oxazepam, clonazepam	GHB, ethanol	RR 20, HR 99, BP 134/77, temp 36.5°, GCS 15			1:33	Medically discharged
M 27	Methylone	Amphetamines, heroin, clonazepam, nitrazepam, methadone	Heroin	RR 10, HR 84, BP 97/63, temp 35.5°, GCS 9			9:42	Medically discharged
M 25	N,N-dimethyl-MDA	MDMA	MDMA, ethanol	RR 18, HR 118, temp 36.9°, GCS 15	Agitation		2:17	Medically discharged
M 42	JWH-210	Heroin	Heroin, amphetamines	HR 81, BP 112/60, temp 39.5°	Hyperthermia	Treated for abscess in groin	372:21	Admitted ICU
F 31	JWH-210	Amphetamines, diazepam, clonazepam, alprazolam	GHB	RR 16, HR 55, BP 120/65, temp 35.7°, GCS 13	Dyspnoea, anxiety, agitation		2:09	Self discharge
M 29	AM-2201	Amphetamines, clonazepam, alprazolam	Amphetamines, opioid	RR 12, HR 89, BP 131/81, temp 36.5°, GCS 15	Dyspnoea, headache, palpitations, chest pain, arrhythmia, CK 1275		49:01	Admitted ICU
F 24	AM-2201	Heroin, diazepam	Heroin	RR 26, HR 37, BP 114/49, temp 35.7°, GCS 15	Dyspnoea, anxiety, palpitations		36:09	Admitted ICU
F 31	5-EAPB	Amphetamines, clonazepam, heroin, fentanyl	GHB	RR 8, HR 51, BP 86/42, temp 34.6°, GCS 3	Dyspnoea, hypotension	Intubated, propofol	10:49	Admitted ICU

4-MA, DMT, methylone and N,N-dimethyl-MDA cases found at the outpatient clinic. JWH-210, AM-2201 and 5-EAPB cases found among the hospital patients.

4-MA: 4-methylamphetamine, DMT: dimethyltryptamine, N,N-dimethyl-MDA: N,N-dimethyl-3,4-methylenedioxyamphetamine, JWH-210: 4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone, AM-2201: (1-(5-fluoropentyl)-3-(1-naphthoyl)indole), 5-EAPB: 5-(2-ethylaminopropyl)benzofuran, THC: tetrahydrocannabinol, MDMA: 3,4-methylenedioxymethamphetamine, GHB: gamma-hydroxybutyrate, RR: respiratory rate, HR: heart rate, BP: blood pressure, GCS: Glasgow coma scale score, CK: creatine kinase, ICU: intensive care unit.

Figure 1. Cases included and samples collected and analysed



Supplementary Table 1	Cut-off values for the different substances (nM)										
Substance	**Blood**	**Oral fluid**	**Substance**	**Blood**	**Oral fluid**	**Substance**	**Blood**	**Oral fluid**	**Substance**	**Blood**	**Oral fluid**
Diazepam	200	0.70	5F-APINACA	0.10	0.10	2,5-DMA	20	2.0	Ethylphenidate	20	2.0
Nordiazepam	200	0.70	5F-PB-22	0.20	0.10	25I-NBOMe	20	2.0	AH-7921	20	2.0
Oxazepam	600	1.0	AM-2201	0.20	0.025	25C-NBOMe	20	2.0	Carfentanyl	20	2.0
Alprazolam	10	1.0	AM-2233	0.20	0.050	2C-B	20	n.a.	1-benzylpiperazine	20	2.0
Clonazepam	4.0	1.0	AM-694	0.20	0.10	2C-C	20	2.0	mCPP	20	2.0
7-aminoclonazepam	n.a.	1.0	APINACA	0.20	0.050	2C-E	20	2.0	TFMPP	20	2.0
Nitrazepam	50	1.0	BB-22	0.20	0.10	2C-I	20	2.0	5-MeO-DMT	20	2.0
7-aminonitrazepam	n.a.	1.0	HU-210	2.0	1.0	2C-P	20	2.0	alpha-methyltryptamine	20	2.0
Flunitrazepam	5.0	1.0	JWH-015	0.20	0.025	2C-T-2	20	2.0	DMT	20	2.0
7-aminoflunitrazepam	n.a.	1.0	JWH-018	0.20	0.025	2C-T-7	20	2.0	Desomorphine	20	n.a.
Bromazepam	50	4.0	JWH-019	0.20	0.025	2-FA	20	2.0	DMAA	20	2.0
Lorazepam	30	2.0	JWH-073	0.50	0.025	3-FA	20	2.0	Homoamphetamine	20	2.0
Fenazepam	5.0	1.0	JWH-081	0.50	0.025	4-FA	20	2.0	Pentedrone	20	2.0
Etizolam	20	2.0	JWH-122	0.50	0.025	4-FMA	20	2.0	Salvinorin A	20	2.0
Diclazepam	20	2.0	JWH-200	0.20	0.050	4-MA	20	2.0			
Flubromazepam	20	2.0	JWH-203	0.20	0.10	4-MMA	20	2.0			
Pyrazolam	20	2.0	JWH-210	0.10	0.10	4-MTA	20	2.0			
Zopiclone	20	2.0	JWH-250	0.50	0.025	BDB	20	2.0			
Zolpidem	70	2.0	JWH-251	0.20	0.10	Bromo-Dragonfly	20	2.0			
Amphetamine	200	17	MAM-2201	0.20	0.025	bk-MBDB	20	2.0			
Methamphetamine	200	17	PB-22	0.20	0.050	DOB	20	2.0			
MDMA	200	20	RCS-4-C4	0.20	0.050	DOI	20	2.0			
MDEA	200	n.a.	RSC-4	0.50	0.10	MBDB	20	2.0			
MDA	200	n.a.	RSC-8	0.50	0.10	MDAI	20	2.0			
PMA	50	3.0	STS-135	0.20	0.10	2-AI	20	2.0			
PMMA	50	3.0	UR-144	0.10	0.10	N,N-dimethyl-MDA	20	2.0			
Cocaine	50	2.0	UR-144 degradation product	0.20	0.10	N-OH-MDA	20	2.0			
Benzoylegconine	200	10	URB-754	0.20	0.050	5-EAPB	20	2.0			
THC	2.0	1.0	WIN 55,212-2	0.50	0.025	5-IT	20	2.0			
Morphine	30	7.0	XRL-11	0.20	0.050	6-APB	20	2.0			
M6G	50	n.a.	XRL-11 degradation product	0.20	0.10	MPA	20	2.0			
M3G	50	n.a.				2-MMC	20	2.0			
Codeine	30	3.0				3-MMC	20	2.0			
6-MAM	n.a.	2.0				4-MEC	20	2.0			
Methadone	60	17				alpha-PVP	20	2.0			
Buprenorphine	0.80	3.0				bk-MDDMA	20	2.0			

Tramadol	200	20	Ethylcathinone	20	2.0
Fentanyl	1.0	0.30	MDPV	20	2.0
Ketobemidone	50	n.a.	Methylone	20	2.0
Ketamine	200	n.a.	Methoxetamine	20	2.0
LSD	0.50	0.050	2-DPMP	20	2.0