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PERSPECTIVE

Assessing the toxicological significance of new psychoactive substances in fatalities

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1 | NEW PSYCHOACTIVE SUBSTANCES AND THE EMCDDA

New psychoactive substances make up a broad range of drugs – such as synthetic cannabinoids, stimulants, opioids, benzodiazepines, and hallucinogens – that are not controlled under the international drug control system.[1,2] Usually they are intended as 'legal' replacements for the illicit drug market; while some are also used by small groups who wish to explore them for possible novel experiences and effects.[3-6]

Since 1997, the European Union has had a 3-step legal framework of early warning, risk assessment, and control measures that allows it to rapidly identify and respond to the potential public health risks caused by the appearance of such new substances.[1,2,6-9] The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is responsible for the first two steps in this system, namely operating an early warning system with Europol (the EU Police agency) and conducting risk assessments; while the European Commission, European Parliament, and Council of the European Union are responsible for control measures. As part of this work, the EMCDDA monitors more than 620 new psychoactive substances that have appeared on Europe's drug market over the past 20 years. Data on these substances are collected and reported to the EMCDDA through the EU Early Warning System as well as from the EMCDDA's other monitoring systems.[1,7-9] Most of these substances have been detected over the past decade, with more than 70% detected in the last five years alone – including 66 detected for the first time in 2016.[4] This dramatic growth in the market is also reflected in substantial increases in seizures and other forms of confiscations made by law enforcement over this period.[3-6]

Driving much of this growth is the exploitation of globalization, economic development, and new technologies by entrepreneurs.[3-6] This includes a shift away from a reliance on clandestine laboratories typical of the 1980s and 1990s to commercial operations run by chemical and pharmaceutical companies in China[3-6,10-15] Using business-to-business (B2B) and business-to-consumer (B2C) online marketplaces, as well as their own websites, the companies have secured a global reach for their products; some even offering custom synthesis. The amounts offered for sale range from a few milligrams to tens or even hundreds of kilograms; the purity often claimed to be high. Payments by credit and debit cards, online payment services, or direct bank transfers are accepted. Escrow services and online reputation systems – similar to those found on established e-commerce marketplace and auction websites – may also be used which serve to decrease risk and increases trust between buyer and seller, helping secure sales.[16]

From here substances can be shipped to Europe for as little as €50 or less. The packages easily blend in among the thousands of small packages shipped from China to Europe each day. Larger amounts are shipped by air or sea cargo. Once in Europe, some of the substances are processed into products before packaging and sale. This includes herbal smoking mixtures laced with synthetic cannabinoids – generally known as 'Spice'[9,17] – as well as the more recent development of ready-to-use nasal sprays and e-liquids for use in electronic cigarettes containing fentanyl analogues.[18,19] Some of these substances are sold in specialized high-street shops and their counterparts on the surface web – typically as branded 'legal high' products or 'research chemicals' – as well as on anonymous marketplaces on darknets[20,21] and on the existing illicit market for drugs.[3] Importantly, new substances may be sold as substances and products in their own right as well as passed off to unsuspecting users as illicit drugs such as heroin, cocaine, 'Ecstasy', and benzodiazepines.[22]

While recent indications suggest that certain policy responses can reduce availability in some circumstances – such as measures aimed at reducing the open sale in high-street shops – the overall continued availability is driving greater complexity into the drug problem.[5] Most recently in Europe, this includes an increase in the number and availability of fentanils detected, with some 20 appearing on the market since 2012, including 8 detected for the first time in 2016 alone.[4] Responding to this development, the EMCDDA has strengthened its early warning and response activities to such potent opioids including launching detailed investigations into five of them – acetylfentanyl, acryloylfentanyl, furanylfentanyl, 4-fluoro-isobutyrylfentanyl and

tetrahydrofuranylfentanyl – following reports of more than one hundred deaths between them.[18,19,23,24]

The fallout from the growth in the market for new substances has been an increase in the number of serious adverse events – particularly non-fatal and fatal poisonings.[4,6] As part of its overall response to the growth in the market, the EMCDDA has undertaken a rolling programme of work over the past few years to strengthen key systems which form part of its early warning and response activities. This includes developing interconnected systems that allows it to better identify, understand, prioritize, and respond to public health threats – including a toxicovigilance system, signal management system, open source information monitoring system, and risk communication system. In addition, it has also conducted an increasing number of risk assessments on substances causing particular concerns to the European Union.[25] The toxicovigilance system allows the EMCDDA to identify, manage, understand, and, through other components of the EU Early Warning System and risk assessment process, react to serious adverse events associated with new substances. Much of the initial work has focused on strengthening the detection, reporting, and assessment of serious adverse events reported by the countries which are part of the EU Early Warning System as well as those events identified by the EMCDDA from the scientific and medical literature and other open sources. As part of this work, the EMCDDA is examining a number of complementary approaches to assess and classify the causal role of new psychoactive substances in serious adverse events. This is somewhat similar to the field of pharmacovigilance, where a number of causality assessment/classification systems have been developed for assessing the likelihood of the involvement of a medicine in an adverse event.[26-33]

The aims of this paper are three-fold: (1) highlight the need for such systems; (2) demonstrate one such system, known as the toxicological significance score (TSS), which has been developed to support the risk assessment of new psychoactive substances by allowing the role of specific new psychoactive substances in deaths to be better assessed and classified; and (3) call for further research into such systems in order to help support evidence-based assessments and strengthen the risk assessment of new psychoactive substances.

2 | RISK ASSESSMENT AND TOXICOLOGICAL SIGNIFICANCE

Examination of the individual health risks of a substance is a fundamental requirement of the risk assessment process.[1,9] The toxicological significance of the substance in serious adverse events, especially in deaths, is an essential consideration. Within toxicology, there are various factors that are involved in determining the significance of the presence, absence, or concentration of a substance or metabolites found in biological material following laboratory analysis.[34] These include the circumstances of the case, the nature of the specimen(s) analyzed, the type of analysis performed, the availability and applicability of known concentration ranges, an assessment of tolerance to the substance, the impact of pharmacogenomics, the presence of other substances, stability of the substance in biological material, and the possible *in vitro* or *in vivo* production of the substance (especially in post-mortem situations), in addition to other potential influencing factors such as post-mortem redistribution. When assessing new psychoactive substances in particular, information and evidence pertaining to many of these considerations is not always known. In particular, depending on the nature and developmental history of the new substance, defined or typical concentrations found in varying circumstances are invariably not available. Appropriate pharmacological information is also required to interpret metabolic and pharmacogenomic factors and determine possible drug–drug interactions and the propensity for tolerance.[35] Furthermore, experimental findings would also be required to assess substance instability[36,37] and case findings are needed to assess the occurrence and degree of post-mortem redistribution in fatalities.[38-40]

Whilst it is preferable to assess situations where only the substance under review is involved, this is not always possible with new psychoactive substances due to the common use of multiple drugs. Such polydrug use may be both intentional and unintentional based on the mode of use and occurrence of multi-component products, respectively.[3,41-44] Nevertheless, whilst additional substances may be involved, the toxicological relevance of other drugs may be low due to their pharmacological nature, concentration, and/or their presence in a particular specimen. For example, the presence of a drug in urine only would indicate previous rather than recent use, and absence in the blood would suggest that it was not exerting a pharmacological effect at the time of death or specimen sampling. As such, if drugs are not present in the blood (especially in fatal instances), this reduces their toxicological importance in relation to the new psychoactive substances under review. However, there are exceptions to this, for example in instances where use of a drug has resulted in actions leading to death occurring many hours (or even days) after intake (such as hanging, trauma and other injuries or adverse behaviour).

First, to allow an evidence-based assessment of the toxicological significance of a substance, appropriate laboratory confirmed analytical findings are required. This is a prerequisite in toxicology casework and it is important to have knowledge of the nature of the testing performed (e.g. sensitivity and selectivity) as well as other quality and validation parameters (e.g. precision and accuracy if quantitative).[45] In order to have assurances of detection in biological material, identification using mass spectrometry (invariably coupled to gas or liquid chromatography) is widely accepted,[46] in addition to other techniques (e.g. diode array detection) that may allow elucidation of a particular isomeric form (which is a significant issue for many new psychoactive substances).[47,48] This is important in order to relate particular findings (e.g. clinical and/or pathological) to the particular new psychoactive substance under review, for example, distinguishing 3-methylmethcathinone from 4-methylmethcathinone; mephedrone) which would not be possible even using accurate mass spectrometry due to an identical empirical formula.

3 | ASSESSING TOXICOLOGICAL SIGNIFICANCE

The process for assessing the toxicological significance of new psychoactive substances in fatalities requires the following factors to be considered:

1. Presence, concentration and nature of the new psychoactive substance under review.
2. Presence, concentration and nature of other drugs present (including alcohol).
3. Circumstances of death.
4. Cited cause of death, including pathology findings.
5. Depending on the number of cases, it may be possible to determine typical concentrations in varying circumstances (e.g. direct cause of death, or alternative/unrelated cause of death).

3.1 | Presence, concentration and nature of the new psychoactive substance under review

Assessment of the presence, concentration and nature of the new psychoactive substance under review initially requires evaluation of the analytical evidence (as already mentioned). The relevance of concentration is a primary toxicological consideration whereby invariably the higher the concentration, the more significant the finding due to likely higher probability of toxicity occurring and to a greater degree. In order to assess whether a concentration is relevant or not, it is necessary to have comparative concentration datasets in varying circumstances. For instance, post-mortem concentrations in fatalities with an alternative cause of death (e.g. hanging, gunshot, road traffic accident, trauma from a fall), post-mortem concentrations in drug-related fatalities involving the new psychoactive substance under review and *in life* concentrations (e.g. non-fatal cases and suspected driving under the influence of drugs). The importance of this comparison incorporates the possibility of post-mortem redistribution and other changes that can occur after death which can result in artificially elevated drug concentrations depending on the post-mortem interval, nature of the drug and anatomical site of sampling (whereby blood obtained appropriately from the femoral vein is the preferred source).[38] If such a dataset is not available (as is often the case with new psychoactive substances) knowledge of the pharmacological nature of the new psychoactive substance is required to enable an initial evaluation of the new psychoactive substance's possible actions and effects, including characterization of the new psychoactive substance as, for example, a central nervous system stimulant, depressant and/or hallucinogen, etc. This could also be used to highlight potential interactions with other drugs/substances involved or its impact on pre-existing medical conditions.

3.2 | Presence, concentration and nature of other drugs present (including alcohol)

As toxicological significance can also be assessed through exclusion of other factors, it is necessary to consider the other drugs that may or may not be involved, including ethanol (alcohol). Consequently, as for the new psychoactive substances under review as described above, the same pharmacological aspects and toxicological data need to be assessed. If a medicine is also detected in the biological material, there is likely to be pre-clinical and clinical data available as well as information regarding its actions and expected concentrations with recorded outcomes, including a potential therapeutic range and post-mortem data. However, if another new psychoactive substance is involved then there may not be the same types and volumes of data but its significance can be assessed as above. As such, if no other drugs are detected/involved or even if present the expected contribution to toxicity or death is low, then this provides more weight to the new psychoactive substances under review being significant to the adverse event/death.

3.3 | Circumstances of death

When considering exclusion of other factors in a death, the manner and cause are paramount. There are of course many circumstances in which death can occur. The involvement (or not) of a drug or drugs in the death is a primary consideration. As mentioned elsewhere, whilst hanging or fatal trauma may be the manner of death, it is possible that use of a drug precipitated that event, either through altering the individual's state of mind (including suicidal ideation) or causing behaviours or actions that lead to a trauma (e.g. fall from a height or accidental injury). Aside from suspected drug toxicity or sudden unexplained death, other situations whereby a new psychoactive substances under review may be involved include road traffic incidents, drowning, gunshot wounds (including self-inflicted), asphyxia, fire, etc. In casework it is the role of the toxicologist to assist investigators in determining the potential role of drugs in the death and, in the situation of new psychoactive substances review, the same role applies but as much information as possible is required concerning the circumstances. Nevertheless, even an overview of the manner of death (e.g. hanging or other mechanical suicide) provides information that the death may not be directly related to the new psychoactive substance under review.

3.4 | Cited cause of death, including pathology findings

Based on the circumstances and pathological findings, ultimately the investigating pathologist will have stated a likely cause of death which is recorded by the relevant authority (e.g. Coroner). When determining the cause of death, the concept of exclusion is also applied and the pathologist will look at a wide range of factors, including the deceased's previous medical history as well as macroscopic and microscopic findings post-mortem. As it is invariably not possible to diagnose drug toxicity at autopsy the pathologist equally relies on the toxicological findings but paradoxically in terms of new psychoactive substances, there may be a lack of data to allow such a diagnosis by the pathologist. Therefore, it is possible that the cause of death may be cited as being “unascertained”. Alternatively, and especially if other drugs are involved, the cause of death may be described as, for example, ‘multiple drug toxicity’ or ‘stimulant drug toxicity’ based on any known or suspected action of the drug(s) involved. In these examples, the new psychoactive substance under review may not be specifically mentioned but does not rule out its contribution to the death and should be treated as such when assigning a TSS after performing the assessment and exclusion process above. Primarily, the cited cause of death and pathological findings can be used to determine if there has been an alternative reason for death irrespective of the involvement of the new psychoactive substance.

3.5 | Typical concentrations in varying circumstances

There are often no defined concentration ranges associated with (especially emerging) new psychoactive substances that would constitute 'recreational' or excessive use and corresponding degrees of toxicity and expected outcomes. Furthermore, as outlined above, in fatalities it is necessary to compare post-mortem blood concentrations in cases where new psychoactive substance toxicity is suspected and where there has been an alternative cause of death and/or where new psychoactive substance toxicity is not suspected. Due to the possibility of post-mortem redistribution it is not appropriate to compare *in life* blood concentrations (including driving under the influence) with post-mortem blood concentrations (even if obtained from a peripheral site such as the femoral vein) so collated data in varying circumstances are important if measured concentrations are to be used in the new psychoactive substances review.[49]

4 | TOXICOLOGICAL SIGNIFICANCE SCORE (TSS)

It is possible based on the considerations above for an appropriately qualified and experienced toxicologist to assign a level of significance to the new psychoactive substance under review according to the following scores;

1 = Low, i.e., alternative cause of death.

2 = Medium, i.e., the new psychoactive substance may have contributed to toxicity/death, other drugs present may be more toxicologically significant.

3 = High, i.e., new psychoactive substance cited as cause of death or likely to have contributed to toxicity/death, even in presence of other drugs.

U = Unclassified, i.e., insufficient data to allow assessment.

Although there may be some clear assignations during assessment, there will equally be some situations where scoring may be unclear. In these situations, it may be more appropriate to assign the higher score to ensure sufficient consideration of the new psychoactive substance's involvement or make a critical review of the data to decide whether it is sufficient or not to make an assignation (i.e., unclassified). The following provide worked examples of the classification applied to a new psychoactive substance based on published case report data.[50]

4.1 | Application example: 2-Methoxphenidine

2-Methoxphenidine; 2-MXP; 2-methoxydiphenidine) is a NMDA receptor agonist,[51] similar to ketamine and methoxetamine with purported 'dissociative anaesthetic', hallucinations and potential cardiovascular effects.[14]

4.1.1 | Case 1

Male found dead at home. There was evidence of drug paraphernalia including white powder in clear bags that was later identified to be methoxphenidine (isomer not distinguished). The drug was found to have been purchased over the Internet from a 'research chemical' company. At autopsy, the deceased was found to have an enlarged heart and hypertensive heart disease with no other contributory findings. Cause of death was given as 'methoxyphenidine use and hypertensive heart disease'.

Toxicology findings: post-mortem femoral blood 2-MXP 24.0 mg/L (also present in post-mortem urine). Low concentrations of citalopram, promethazine, lamotrigine and mirtazapine were also found in the blood.

Assessment

Whilst a blood concentration of 24.0 mg/L for ketamine or to an extent, methoxetamine, would be considered to be high there are insufficient comparative data to allow an assessment of the blood 2-MXP concentration in terms of likelihood and degree of any toxicity. The low concentrations of the other drugs do not suggest excessive use of these but it is not possible to rule out possible interactions with 2-MXP. Based on the pre-existing heart disease and potential cardiac effects of 2-MXP along with no other significant pathological findings, 2-MXP can be considered to have likely contributed to death, even in the presence of other drugs and was cited in the cause of death. Toxicological Significance Score of 2-MXP in this instance = 3 (high).

4.1.2 | Case 2

Male found dead at home with a medical history of epilepsy and attention deficit hyperactivity disorder. He had been prescribed levetiracetam, dexamphetamine and diazepam. A sachet labelled methoxphenidine 2 g was found in his pocket. At autopsy, the deceased was found to have a moderately enlarged heart and mild atheroma with no other contributory findings. The cause of death was given as 'probable methoxyphenidine toxicity' due to the absence of any other pathological findings.

Toxicology findings: post-mortem femoral blood 2-MXP 2.0 mg/L (also present in post-mortem urine). Low concentrations of diazepam and quinine were also found in the blood. No levetiracetam or dexamphetamine was detected.

Assessment

Whilst the blood concentration of 2.0 mg/L is lower than that for the other case of suspected 2-MXP toxicity (24.0 mg/L) this does not decrease the likelihood of excessive use, nor support it, solely based on this finding. The low concentrations of the other drugs do not suggest excessive use of these and whilst non-compliance of anti-epileptic medication was considered circumstantial and other evidence excluded this as a factor. With no other significant pathological findings, 2-MXP can be considered to have likely contributed to death, even in presence of other drugs and was cited (albeit on the balance of probability) in the cause of death. Toxicological Significance Score of 2-MXP in this instance = 3 (high).

4.1.3 | Case 3

Male was found dead on a road having jumped or fallen from a road bridge suffering fatal injuries. He had a medical history of schizophrenia. The cause of death was due to multiple injuries following the fall and the inquest conclusion was 'suicide whilst suffering from a mental illness'.

Toxicology findings: post-mortem femoral blood 2-MXP 1.36 mg/L. Low concentration of risperidone also found in the blood.

Assessment

Despite measured concentrations in two cases of suspected 2-MXP toxicity, there are insufficient comparative data to allow an assessment of the blood 2-MXP concentration in terms of likelihood and degree of any toxicity. Nevertheless, the circumstances and stated cause of death conclude that the new psychoactive substance was not the direct cause. Whilst an altered state of mind as a result of 2-MXP use could be a consideration, the pre-existing mental health history is a primary factor. The low concentration of risperidone does not suggest excessive use of this antipsychotic drug. Toxicological Significance Score of 2-MXP in this instance = 1 (low).

In these case situations, if the other (prescription) drugs had been found to be at higher concentrations, it could not be determined with certainty that the new psychoactive substance, 2-MXP would have likely contributed to toxicity/death, even in presence of these other drugs. As such, if that had been the case then a TSS assignation of 2 (medium) would have been more appropriate as 2-MXP may have contributed to toxicity/death but the other drugs present may have been more toxicologically significant.

4.2 | EMCDDA risk assessment

Overall, this classification system allows the evaluation of the toxicological significance of a substance in a death. Systematic assessment of case reports using the described process can be used to produce a matrix of TSS values for new psychoactive substances under review, with quantitative collation of the numbers of cases associated with low, medium and high scores. Specific examples of its application within a risk assessment process include the assessments of 4,4′-DMAR (4,4′-dimethylaminorex; 4-methyl-5-(*p*-tolyl)-4,5-dihydrooxazol-2-amine), MT-45 (1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine), and MDMB-CHIMCA (methyl-(*S*)-2-(1-(cyclohexylmethyl)-1*H*–indole-3-carboxamido)-3,3-dimethylbutanoate) by the EMCDDA.[52-54] For 4,4′-DMAR, out of 31 reported fatalities, 4,4′-DMAR was either the cause of death or was likely to have contributed to death in 23 cases (74%) even in the presence of other substances; in one of these deaths 4,4′-DMAR was the sole drug present. In 8 cases (26%) 4,4′-DMAR may have contributed to toxicity but other substances were present that may have been more toxicologically significant.[52] For MDMB-CHMICA, out of 26 analytically confirmed fatalities with sufficient information, MDMB-CHMICA was reported either as the cause of death or as contributing to death (even in the presence of other substances) in 12 deaths (46%); in 3 of these deaths MDMB-CHMICA was the sole drug present. In 10 deaths (38%) MDMB-CHMICA may have contributed to death but other substances were present that may have been more toxicologically significant. An alternative cause of death was recorded in 4 cases (16%).[54]

4,4′-DMAR: TSS values High (3 score) – 74%, Medium (2 score) – 26%, Low (1 score) – none.

MT-45: TSS values High (3 score) – 68%, Medium (2 score) – 29%, Low (1 score) – 3%.

MDMB-CHMICA: TSS values High (3 score) – 46%, Medium (2 score) – 38%, Low (1 score) – 16%.

5 | FURTHER DISCUSSION

Over the last decade or so, the large increase in the number, type, and availability of new psychoactive substances has led to a range of challenges for public health policy and practice. With an assessment of risk to health being essential and central factor in decision making, it is imperative that some form of mechanism exists such that serious adverse events and other issues are properly evaluated in relation to the potential role of a substance under review. An appropriate appraisal of toxicological significance is a primary part of this process. The proposed TSS is one approach that may assist, a primary strength being based on the same principles as those routinely used in post-mortem toxicology for assessing the potential role of drugs in a death. The approach also allows a 'quantitative' presentation of significance, for example in relation to the proportion of cases at each level of significance/contribution but it may also be used for relative comparison to other substances previously reviewed. This is achieved by providing a consistent approach and subsequent output that does not necessarily require toxicological expertise to understand the conclusions and relative harm. There are limitations, however, much of which is dependent on the individual serious adverse events, including the circumstances of death (which may not be straightforward or fully known), as well as potentially unknown or complicated pharmacology of the substance in question in addition to any other drugs involved (which may also involve unpredictable drug–drug interactions). Moreover, important analytical challenges and considerations exist around the measurement of new psychoactive substances, including lack of availability of reference material and validation requirements.[55] Therefore, even if measured, without a sufficient dataset, discrete concentrations may be of little assistance, notwithstanding additional factors that may adversely affect the findings such as instability of some substances resulting in lower concentrations, hence not reflecting the true situation. Finally, the approach is focused on deaths within serious adverse events but non-fatal intoxications and other situations (e.g. drug driving) where a new psychoactive substance may have contributed are also important and necessary aspects of substance review. These limitations may lead to an underestimation of significance or unintentional incomplete evaluation of the factors but this does not detract from the strengths and purpose of the approach.

6 | CONCLUSION

In this paper, we present a system that allows the toxicological significance of new psychoactive substances to be assessed for the purposes of risk assessment — an essential component in helping characterize the potential health and social risks of a substance.[1,2,9,25] This, in turn, can help inform public health policy and practice in respect to risk reduction – including informing risk communication activities, harm reduction, as well as decision making such as consideration of the need for control measures. Causality assessment/

classification systems all have limitations[26-33] but there is a need for systematic, reproducible and transparent approaches.[56,57] Whilst there is equally a need for validation and additional research of this particular approach, the TSS addresses many of the challenges in the assessment of new substances and provides a basis for further discussion and future studies.

REFERENCES

1. European Council. Council decision 2005/387/JHA of 10 may 2005 on the information exchange, risk-assessment and control of new psychoactive substances. 2005. Available at: http://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32005D0387
2. European Commission. Regulation (EC) no. 1920/2006 of the European Parliament and of the Council of 12 December 2006 on the European monitoring Centre for Drugs and Drug Addiction (recast). 2006. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006R1920
3. European Monitoring Centre for Drugs and Drug Addiction. EU drug markets report. In depth analysis. 2016. Available at: https://dx.doi.org/10.2810/219411
4. European Monitoring Centre for Drugs and Drug Addiction. European drug report: Trends and developments. 2016. Available at: https://dx.doi.org/10.2810/04312
5. Brandt S, King L, Evans-Brown M. The new drug phenomenon. *Drug Test Anal*. 2014;6:587
6. Evans-Brown M, Sedefov R. New psychoactive substances: Driving greater complexity into the drug problem. *Addiction*. 2017;112:36
7. European Monitoring Centre for Drugs and Drug Addiction. Action on new drugs. 2017. Available at: http://www.emcdda.europa.eu/activities/action-on-new-drugs
8. European Monitoring Centre for Drugs and Drug Addiction. Early-warning system on new psychoactive substances. Operating guidelines. 2007. Available at: http://www.emcdda.europa.eu/system/files/publications/449/EWSguidelines2_98082.pdf
9. European Monitoring Centre for Drugs and Drug Addiction. Risk assessment of new psychoactive substances. Operating guidelines. 2009. Available at: http://www.emcdda.europa.eu/system/files/publications/557/RiskGuidelines2010_203593.pdf
10. European Monitoring Centre for Drugs and Drug Addiction. Understanding the 'Spice' phenomenon. 2009. Available at: http://www.emcdda.europa.eu/system/files/publications/537/Spice-Thematic-paper-final-version.pdf
11. Jack H.. The story of Spice. *Financial Times*, 2009.
12. Morris H. Interview with a ketamine chemist. *Vice*. 2011. Available at: http://www.vice.com/en_uk/read/interview-with-ketamine-chemist-704-v18n2
13. Morris H. Criminal chlorination. An interview with a clandestine chemist. *Vice*. 2012. Available at: http://www.vice.com/read/criminal-chlorination-0000350-v19n9
14. Morris H, Wallach J. From PCP to MXE: A comprehensive review of the non-medical use of dissociative drugs. *Drug Test Anal*. 2014;6:614
15. Halford. A glimpse inside the sophisticated world of synthetic cannabinoids. *Chemical & Engineering News*. 2015. Available at: http://cen.acs.org/articles/93/web/2015/04/Glimpse-Inside-Sophisticated-World-Synthetic.html
16. Masum H, Tovey M (Eds). *The Reputation Society. How Online Opinions are Reshaping the Offline World*. Cambridge, MA, USA: MIT Press; 2012.
17. European Monitoring Centre for Drugs and Drug Addiction. Synthetic cannabinoids in Europe. 2017. Available at: http://www.emcdda.europa.eu/topics/pods/synthetic-cannabinoids
18. European Monitoring Centre for Drugs and Drug Addiction. EMCDDA–Europol Joint report on a new psychoactive substance: *N*-(1-phenethylpiperidin-4-yl)-*N*-phenylacrylamide (acryloylfentanyl). 2017. Available at: https://dx.doi.org/10.2810/87713
19. Ujváry I, Jorge R, Christie R, Le Ruez T, Danielsson HV, Kronstrand R, Elliott S, Gallegos A, Sedefov R, Evans-Brown M. Acryloylfentanyl, a recently emerged new psychoactive substance: A comprehensive review. *Forensic Tox*. 2017 (in press). https://dx.doi.org/10.1007/s11419-017-0367-8
20. Christin N. Traveling the silk road: A measurement analysis of a large anonymous online marketplace. In *Proceedings of the 22nd International World Wide Web Conference (WWW'13)*, pp. 213–224. Rio de Janeiro, Brazil. May 2013. Available at: https://www.andrew.cmu.edu/user/nicolasc/publications/Christin-WWW13.pdf
21. European Monitoring Centre for Drugs and Drug Addiction. The internet and drug markets. 2016. Available at: https://dx.doi.org/10.2810/324608
22. Quintana P, Ventura M, Grifell M, et al. The hidden web and the fentanyl problem: Detection of ocfentanil as an adulterant in heroin. *Int J Drug Policy*. 2016;40:78
23. European Monitoring Centre for Drugs and Drug Addiction. EMCDDA–Europol Joint report on a new psychoactive substance: *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl] acetamide (acetylfentanyl). 2016. Available at: https://dx.doi.org/10.2810/890694
24. European Monitoring Centre for Drugs and Drug Addiction. *EMCDDA–Europol Joint Report on a new psychoactive substance: N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]-furan-2-carboxamide (furanylfentanyl)*. Luxembourg: Publications Office of the European Union; 2017.
25. European Monitoring Centre for Drugs and Drug Addiction. Risk assessments. 2010. Available at: http://www.emcdda.europa.eu/html.cfm/index16776EN.html
26. Aronson JK. In: Talbot J, Aronson JK, eds. *Stephens' Detection and Evaluation of Adverse Drug Reactions: Principles and Practice*. Chichester, UK: John Wiley & Sons Ltd; 2012:1-119.
27. Aronson JK. In: Andrews EB, Moor N, eds. *Mann's Pharmacovigilance*. Chichester, UK: John Wiley & Sons Ltd; 2014:121-138.
28. Jones JK. E. Kingery. In: Andrews EB, Moor N, eds. *Mann's Pharmacovigilance*. Chichester, UK: John Wiley & Sons Ltd; 2014:319-330.
29. Uppsala Monitoring Centre. The use of the WHO-UMC system for standardised case causality assessment. 1997. Available at: http://www.who.int/medicines/areas/quality_safety/safety_efficacy/WHOcausality_assessment.pdf
30. Agbabiaka TB, Savović J, Ernst E. Methods for causality assessment of adverse drug reactions: A systematic review. *Drug Saf*. 2008;31:21
31. Arimone Y, Miremont-Salamé G, Haramburu F, et al. Inter-expert agreement of seven criteria in causality assessment of adverse drug reactions. *Br J Clin Pharmacol*. 2007;64:482
32. Théophile H, André M, Miremont-Salamé G, Arimone Y, Bégaud B. Comparison of three methods (an updated logistic probabilistic method, the Naranjo and Liverpool algorithms) for the evaluation of routine pharmacovigilance case reports using consensual expert judgement as reference. *Drug Saf*. 2013;36(10):1033
33. Edwards RI. Causality assessment in pharmacovigilance: Still a challenge. *Drug Saf*. 2017;40:365
34. Cooper GA, Paterson S, Osselton MD. United Kingdom and Ireland Association of Forensic Toxicologists. The United Kingdom and Ireland Association of Forensic Toxicologists Forensic toxicology laboratory guidelines. *Sci Justice*. 2010;50:166
35. Green AR, Nutt DJ. Pharmacology should be at the centre of all preclinical and clinical studies on new psychoactive substances (recreational drugs). *J Psychopharmacol*. 2014;28:711
36. Soh YN, Elliott S. An investigation of the stability of emerging new psychoactive substances. *Drug Test Anal*. 2014;6:696
37. Johnson RD, Botch-Jones SR. The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *J Anal Toxicol*. 2013;37:51

38. Pounder DJ, Jones GR. Post-mortem drug redistribution - a toxicological nightmare. *Forensic Sci Int*. 1990;45:253
39. Han E, Kim E, Hong H, et al. Evaluation of postmortem redistribution phenomena for commonly encountered drugs. *Forensic Sci Int*. 2012;219:265
40. De Letter EA, Stove CP, Lambert WE, Piette MH. Post-mortem (re)distribution of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"): Human and animal data. *Curr Pharm Biotechnol*. 2010;11:453
41. Hondebrink L, Nugteren-van Lonkhuyzen JJ, Van Der Gouwe D, Brunt TM. Monitoring new psychoactive substances (new psychoactive substances) in the Netherlands: Data from the drug market and the poisons information Centre. *Drug Alcohol Depend*. 2015;147C:109
42. Maciów-Głąb M, Rojek S, Kula K, Kłys M. 'new designer drugs' in aspects of forensic toxicology. *Arch Med Sadowej Kryminol*. 2014;64:20
43. Zamengo L, Frison G, Bettin C, Sciarrone R. Understanding the risks associated with the use of new psychoactive substances (new psychoactive substances): High variability of active ingredients concentration, mislabelled preparations, multiple psychoactive substances in single products. *Toxicol Lett*. 2014;229:220
44. Zuba B. Byrska. *Prevalence and co-existence of active components of 'legal highs' Drug Test Anal*. 2013;5:420
45. Peters FT, Drummer OH, Musshoff F. Validation of new methods. *Forensic Sci Int*. 2007;165:216
46. Favretto D, Pascali JP, Tagliaro F. New challenges and innovation in forensic toxicology: Focus on the "new psychoactive substances". *J Chromatogr A*. 2013;1287:84
47. Elliott S, Smith C. Investigation of the first deaths in the United Kingdom involving the detection and quantitation of the piperazines BZP and 3-TFMPP. *J Anal Toxicol*. 2008;32:172
48. Elliott SP, Brandt SD, Freeman S, Archer RP. AMT (3-(2-aminopropyl) indole) and 5-IT (5-(2-aminopropyl)indole): An analytical challenge and implications for forensic analysis. *Drug Test Anal*. 2013;5:196
49. Elliott S, Evans J. A 3-year review of new psychoactive substances in casework. *Forensic Sci Int*. 2014;243:55
50. Elliott S, Brandt S, Wallach J, Morris H, Kavanagh P. First reported fatalities associated with the 'research chemical' 2-methoxydiphenidine (MXP). *J Anal Toxicol*. 2015;39:287
51. Wallach J, Kang H, Colestock T, et al. Pharmacological investigations of the dissociative 'legal highs' diphenidine, methoxphenidine and analogues. *PLoS One*. 2016;11: e0157021
52. European Monitoring Centre for Drugs and Drug Addiction. Report on the risk assessment of 4,4′-DMAR in the framework of the Council decision on new psychoactive substances. 2015. Available at: https://dx.doi.org/10.2810/58185
53. European Monitoring Centre for Drugs and Drug Addiction. Report on the risk assessment of MT-45 in the framework of the Council decision on new psychoactive substances. 2015. Available at: https://dx.doi.org/10.2810/59253
54. European Monitoring Centre for Drugs and Drug Addiction. Report on the risk assessment of methyl 2-[[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-CHMICA) in the framework of the Council decision on new psychoactive substances. 2017. Available at: https://dx.doi.org/10.2810/964776
55. Gerostamoulos D, Elliott S, Walls HC, Peters FT, Lynch M, Drummer OH. To measure or not to measure? That is the NPS question. *J Anal Toxicol*. 2016;40:318
56. Danenberg LA, Sorge W, Wieniawski S, Elliott L, Amato WK. Scholten. Modernizing methodology for the WHO assessment of substances for the international drug control conventions. *Drug Alcohol Depend*. 2013;131:175
57. Nutt DJ, King LA, Phillips LD. Independent scientific committee on drugs. Drug harms in the UK: A multicriteria decision analysis. *Lancet*. 2010;376:1558

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